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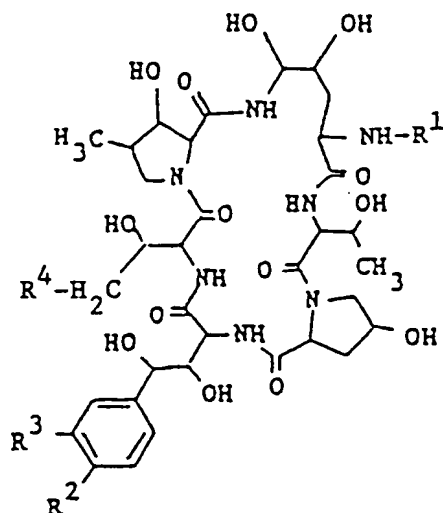
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(54) Cyclic polypeptide with antibiotic activity, process for its preparation and pure culture of a Coelomycetes strain.

(57) A polypeptide compound of the following general formula :

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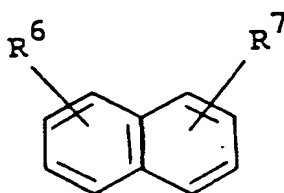
wherein

- R<sup>1</sup> is hydrogen or acyl group,  
 R<sup>2</sup> is hydroxy or acyloxy,  
 R<sup>3</sup> is hydrogen or hydroxysulfonyloxy, and  
 R<sup>4</sup> is hydrogen or carbamoyl,

with proviso that

- (i) R<sup>2</sup> is acyloxy, when R<sup>3</sup> is hydrogen, and  
 (ii) R<sup>1</sup> is not palmitoyl, when R<sup>2</sup> is hydroxy,  
 R<sup>3</sup> is hydroxysulfonyloxy and  
 R<sup>4</sup> is carbamoyl,

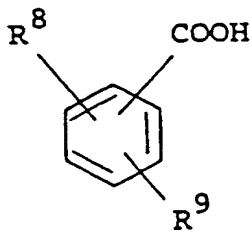
and a pharmaceutically acceptable salt thereof, processes for their preparation and pharmaceutical compositions comprising them. The invention relates also to intermediates of the formula



wherein

- R<sup>6</sup> is (C<sub>4</sub>-C<sub>6</sub>)alkoxy, higher alkoxy or higher alkenyloxy, and  
 R<sup>7</sup> is -COOH or -SO<sub>3</sub>H,

or its reactive derivative at the carboxy group or a salt thereof and



Wherein

- R<sup>8</sup> is 1 to 4 halogen, and

$R^9$  is lower alkoxy which has one or more halogen, higher alkoxy which has one or more halogen, or its reactive derivative at the carboxy group or a salt thereof. The invention also relates to a biologically pure culture of the microorganism *Coelomycetes* strain F-11899 (FERM BP-2635).

The present invention relates to new polypeptide compound and a pharmaceutically acceptable salt thereof.

More particularly, it relates to new polypeptide compound and a pharmaceutically acceptable salt thereof, which have antimicrobial activities (especially antifungal activities), to a process for preparation thereof, to pharmaceutical composition comprising the same, and to a method for treating or preventing infectious diseases in human being or animals.

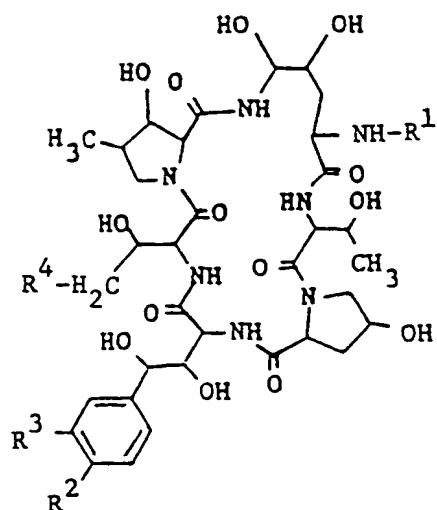
Accordingly, one object of the present invention is to provide the polypeptide compound and a pharmaceutically acceptable salt thereof, which are highly active against a number of pathogenic microorganisms in human being and animals.

Another object of the present invention is to provide a process for the preparation of the polypeptide compound and a salt thereof.

A further object of the present invention is to provide a pharmaceutical composition comprising, as an active ingredient, said polypeptide compound or a pharmaceutically acceptable salt thereof.

Still further object of the present invention is to provide a method for treating or preventing infectious diseases caused by pathogenic microorganisms, which comprises administering said polypeptide compound to human being or animals.

The object polypeptide compound of the present invention is novel and can be represented by the following general formula [I] :



[I]

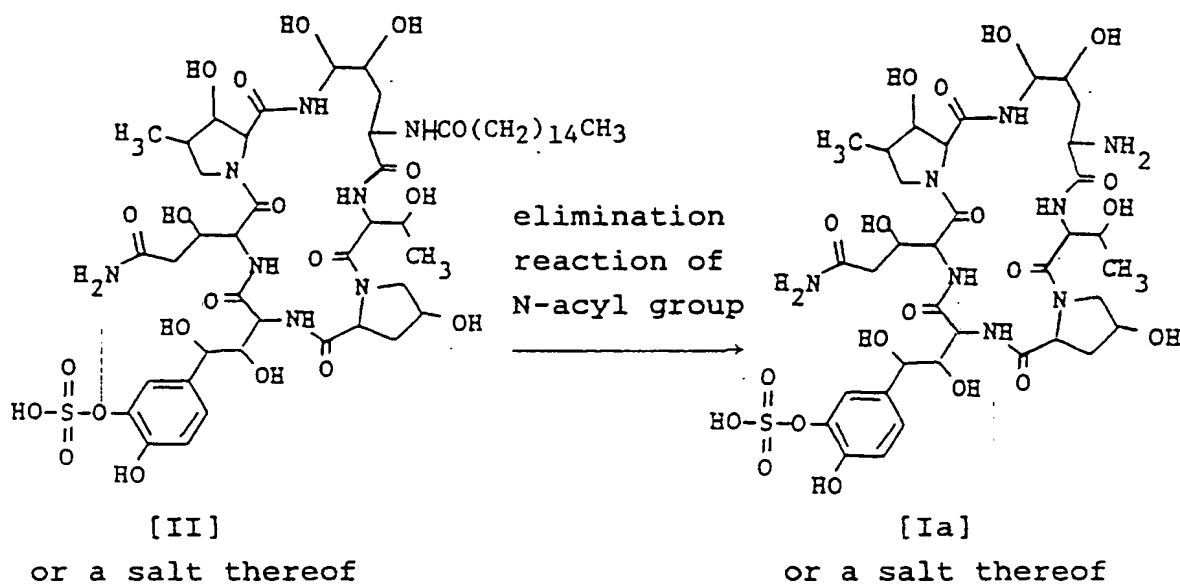
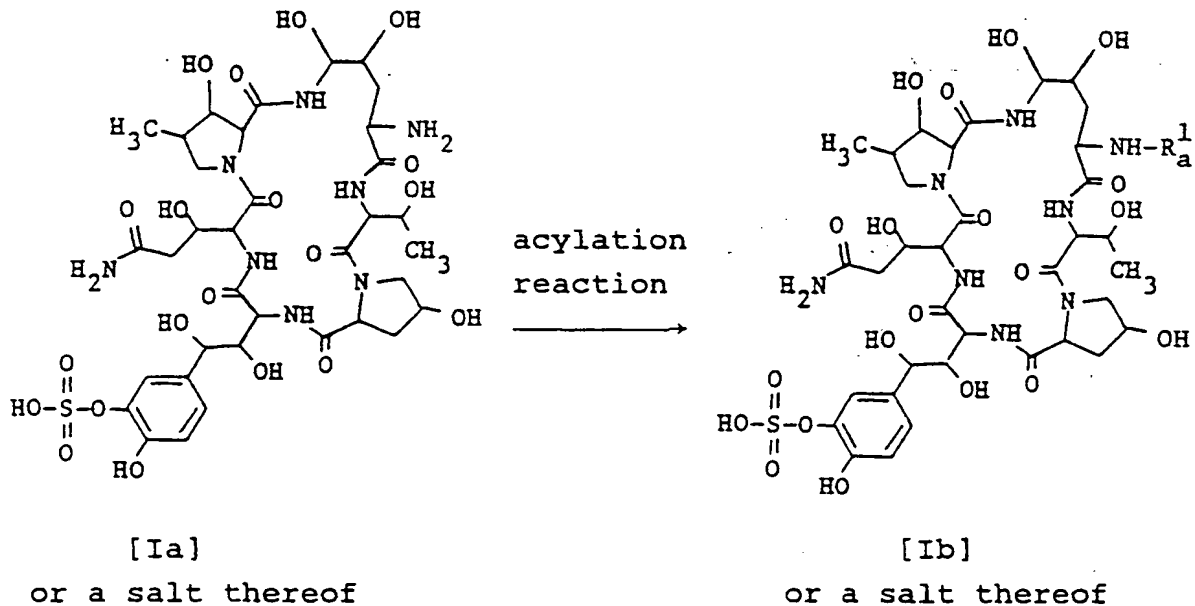
wherein

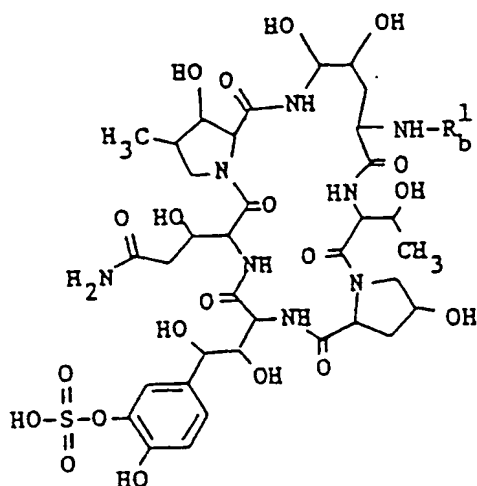
- R<sup>1</sup> is hydrogen or acyl group,  
R<sup>2</sup> is hydroxy or acyloxy,  
R<sup>3</sup> is hydrogen or hydroxysulfonyloxy, and  
R<sup>4</sup> is hydrogen or carbamoyl,

with proviso that

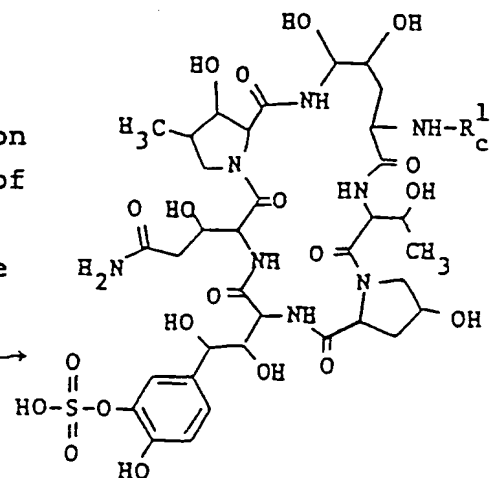
- (i) R<sup>2</sup> is acyloxy, when R<sup>3</sup> is hydrogen, and  
(ii) R<sup>1</sup> is not palmitoyl, when R<sup>2</sup> is hydroxy,  
R<sup>3</sup> is hydroxysulfonyloxy and  
R<sup>4</sup> is carbamoyl.

The polypeptide compound [I] of the present invention can be prepared by the processes as illustrated in the following schemes.

Process 1Process 2

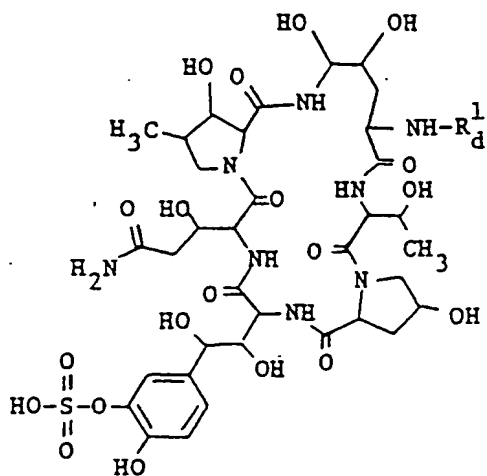
Process 3

elimination  
reaction of  
amino  
protective  
group

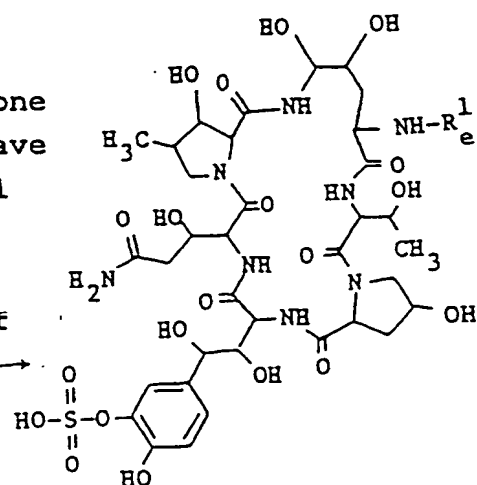


[Ic]  
or a salt thereof

[Id]  
or a salt thereof

Process 4

Pyridinethione  
which may have  
higher alkyl  
[III]  
or a salt  
thereof



[Ie]  
or a salt thereof

[If]  
or a salt thereof

Process 5

5

10

15

20

25

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wherein

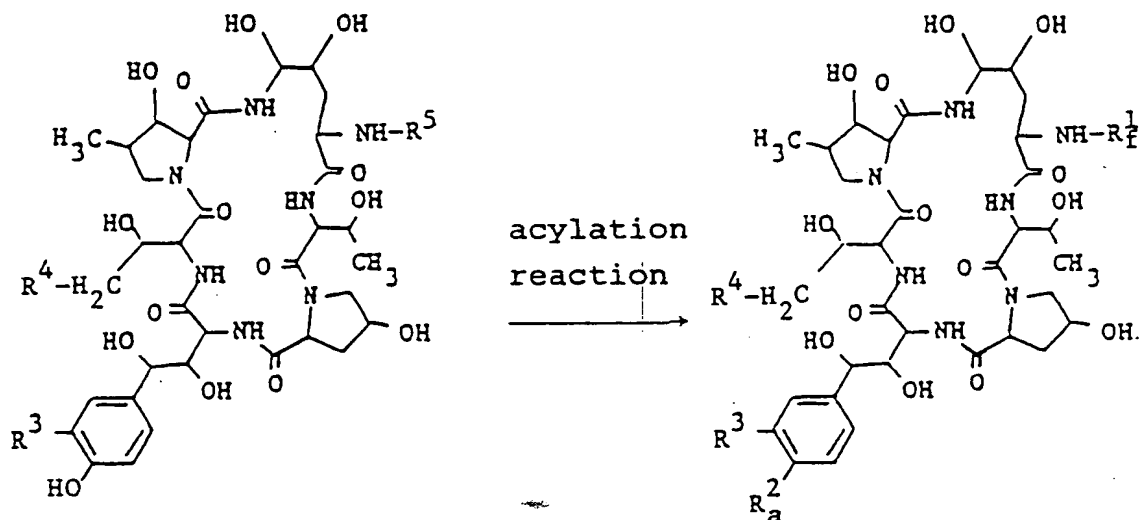
- $R^3$  and  $R^4$  are each as defined above,  
 $R_a^1$  is acyl group exclusive of palmitoyl,  
 $R_b^1$  is ar(lower)alkanoyl which has higher alkoxy and protected amino,  
 $R_c^1$  is ar(lower)alkanoyl which has higher alkoxy and amino,  
 $R_d^1$  is halo(lower)alkanoyl,  
 $R_e^1$  is pyridylthio(lower)alkanoyl which may have higher alkyl,  
 $R_f^1$  is acyl group,  
 $R_a^2$  is acyloxy, and  
 $R^5$  is acyl group.

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[IV]

[Ig]

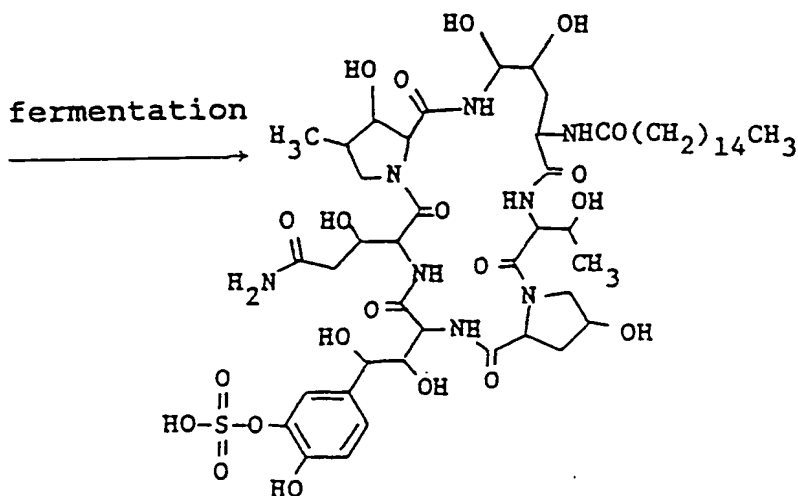
or a salt thereof

or a salt thereof

The starting compound [II] or a salt thereof is novel and can be prepared by the following fermentation process.

Process A

A strain belonging to the Coleophoma which is capable of producing the compound [II] or a salt thereof



[II]  
or a salt thereof

Some of the starting compound [IV] are novel and can be prepared according to the aforesaid Process 1 to 4.

Suitable pharmaceutically acceptable salt of the object compound [I] is conventional non-toxic mono or di salts and include a metal salt such as an alkali metal salt [e.g. sodium salt, potassium salt, etc.] and an alkaline earth metal salt [e.g. calcium salt, magnesium salt, etc.], an ammonium salt, an organic base salt [e.g. trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, dicyclohexylamine salt, N,N-dibenzylethylenediamine salt, etc.] an organic acid addition salt [e.g. formate, acetate, trifluoroacetate, maleate, tartrate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.], an inorganic acid addition salt [e.g. hydrochloride, hydrobromide, hydroiodide, sulfate, phosphate, etc.], a salt with an amino acid [e.g. arginine salt, aspartic acid salt, glutamic acid salt, etc.], and the like.

In the above and subsequent description of this specification, suitable examples of the various definitions are explained in detail as follows :

The term "lower" is intended to mean 1 to 6 carbon atom(s), unless otherwise indicated.

The term "higher" is intended to mean 7 to 20 carbon atoms, unless otherwise indicated.

Suitable "acyl group" may be aliphatic acyl, aromatic acyl, heterocyclic acyl, arylaliphatic acyl and heterocyclic-aliphatic acyl derived from carboxylic acid, carbonic acid, carbamic acid, sulfonic acid, and the like.

Suitable example of the "acyl group" thus explained may be :

lower alkanoyl [e.g. formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, hexanoyl, pivaloyl, etc.] which may have one or more (preferably 1 to 3) suitable substituent(s) such as halogen (e.g. fluoro, chloro, bromo, iodo); aryl (e.g. phenyl, naphthyl, anthryl, etc.) which may have one or more (preferably 1 to 3) suitable substituent(s) like hydroxy, higher alkoxy as explained below, aforesaid aryl, or the like; lower alkoxy as explained below; amino; protected amino, preferably, acylamino such as lower alkoxycarbonylamino (e.g. methoxycarbonylamino, ethoxycarbonylamino, propoxycarbonylamino, butoxycarbonylamino, t-butoxycarbonylamino, pentyloxycarbonylamino, hexyloxycarbonylamino, etc.); or the like; di(lower)alkylamino (e.g. dimethylamino, N-methylethylamino, diethylamino, N-propylbutylamino, dipentylamino, dihexylamino, etc.); lower alkoxyimino (e.g. methoxyimino, ethoxyimino, propoxyimino, butoxyimino, t-butoxyimino, pentyloxymino, hexyloxymino, etc.); ar(lower)alkoxyimino such as phenyl(lower)alkoxyimino (e.g. benzyloxyimino, phenethyloxyimino, benzhydryloxyimino, etc.) which may have one or more (preferably 1 to 3) suitable substituent(s) like higher alkoxy as explained below, or the like; heterocyclicthio, preferably, pyridylthio, which may have one or more (preferably 1 to 3) suitable substituent(s) like higher alkyl (e.g. heptyl, octyl, 2-ethylhexyl, nonyl, decyl, 3,7-dimethyloctyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, 3-methyl-10-



ethyl dodecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, icosyl, etc.), or the like; heterocyclic group (e.g. thienyl, imidazolyl, pyrazolyl, furyl, tetrazolyl, thiazolyl, thiadiazolyl, etc.) which may have one or more (preferably 1 to 3) suitable substituent(s) like amino, aforesaid protected amino, aforesaid higher alkyl, or the like; or the like;

5 higher alkanoyl [e.g. heptanoyl, octanoyl, nonanoyl, decanoyl, undecanoyl, lauroyl, tridecanoyl, myristoyl, pentadecanoyl, palmitoyl, 10,12-dimethyltetradecanoyl, heptadecanoyl, stearoyl, nonadecanoyl, icosanoyl, etc.];

lower alkenoyl [e.g. acryloyl, methacryloyl, crotonoyl, 3-pentenoyl, 5-hexenoyl, etc.] which may have one or more (preferably 1 to 3) suitable substituent(s) such as aforesaid aryl which may have one or more (preferably 1 to 3) suitable substituent(s) like higher alkoxy as explained below, or the like, or the like;

10 higher alkenoyl [e.g. 4-heptenoyl, 3-octenoyl, 3,6-decadienoyl, 3,7,11-trimethyl-2,6,10-dodecatrienoyl, 4,10-heptadecadienoyl, etc.];

lower alkoxy carbonyl [e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl, t-butoxycarbonyl, pentyloxycarbonyl, hexyloxycarbonyl, etc.];

15 higher alkoxy carbonyl [e.g. heptyloxycarbonyl, octyloxycarbonyl, 2-ethylhexyloxycarbonyl, nonyloxycarbonyl, decyloxycarbonyl, 3,7-dimethyloctyloxycarbonyl, undecyloxycarbonyl, dodecyloxycarbonyl, tridecyloxycarbonyl, tetradecyloxycarbonyl, pentadecyloxycarbonyl, 3-methyl-10-ethyl dodecyloxycarbonyl, hexadecyloxycarbonyl, heptadecyloxycarbonyl, octadecyloxycarbonyl, nonadecyloxycarbonyl, icosyloxycarbonyl, etc.];

20 aryloxycarbonyl [e.g. phenoxycarbonyl, naphthylloxycarbonyl, etc.];

arylglyoxyloyl [e.g. phenylglyoxyloyl, naphthylglyoxyloyl, etc.];

ar(lower)alkoxy carbonyl which may have one or more suitable substituent(s) such as phenyl(lower)alkoxy carbonyl which may have nitro or lower alkoxy [e.g. benzyloxycarbonyl, phenethylloxycarbonyl, p-nitrobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, etc.];

25 lower alkylsulfonyl [e.g. methylsulfonyl, ethylsulfonyl, propylsulfonyl, isopropylsulfonyl, pentylsulfonyl, butylsulfonyl, etc.];

arylsulfonyl [e.g. phenylsulfonyl, naphthylsulfonyl, etc.] which may have one or more (preferably 1 to 3) suitable substituent(s) such as lower alkyl as explained below, higher alkoxy as explained below, or the like;

30 ar(lower)alkylsulfonyl such as phenyl(lower)alkylsulfonyl [e.g. benzylsulfonyl, phenethylsulfonyl, benzhydrylsulfonyl, etc.], or the like;

aroyl [e.g. benzoyl, naphthoyl, anthrylcarbonyl, etc.] which may have one or more (preferably 1 to 5) suitable substituent(s) such as aforesaid halogen; lower alkyl (e.g. methyl, ethyl, propyl, butyl, t-butyl, pentyl, hexyl, etc.); aforesaid higher alkyl; lower alkoxy (e.g. methoxy, ethoxy, propoxy, butoxy, t-butoxy, pentyloxy, hexyloxy, etc.) which may have one or more (preferably 1 to 10) suitable substituent(s) like aforesaid lower alkoxy, aforesaid halogen, aforesaid aryl, or the like; higher alkoxy (e.g. heptyloxy, octyloxy, 2-ethylhexyloxy, nonyloxy, decyloxy, 3,7-dimethyloctyloxy, undecyloxy, dodecyloxy, tridecyloxy, tetradecyloxy, pentadecyloxy, 3-methyl-10-ethyl dodecyloxy, hexadecyloxy, heptadecyloxy, octadecyloxy, nonadecyloxy, icosyloxy, etc.) which may have one or more (preferably 1 to 17) suitable substituent(s) like aforesaid halogen; higher alkenyloxy (e.g. 3-heptenyloxy, 7-octenyloxy, 2,6-octadienyloxy, 5-nonenyloxy, 1-decenyloxy, 3,7-dimethyl-6-octenyloxy, 3,7-dimethyl-2,6-octadienyloxy, 8-undecenyloxy, 3,6,8-dodecatrienyloxy, 5-tridecenyloxy, 7-tetradecenyloxy, 1,8-pentadecadienyloxy, 15-hexadecenyloxy, 11-heptadecenyloxy, 7-octadecenyloxy, 10-nonadecenyloxy, 18-icosenyloxy, etc.); carboxy; aforesaid aryl which may have one or more (preferably 1 to 3) suitable substituent(s) like aforesaid higher alkoxy; aryloxy (e.g. phenoxy, naphthoxy, anthryloxy, etc.) which may have one or more (preferably 1 to 3) suitable substituent(s) like aforesaid lower alkoxy, or aforesaid higher alkoxy; or the like; or the like.

45 In said "acyl group", the preferred one may be lower alkanoyl; halo(lower)alkanoyl;

ar(lower)alkanoyl which may have one or more (preferably 1 to 3) hydroxy, lower alkoxy, higher alkoxy, aryl, amino, protected amino, di(lower)alkylamino, lower alkoxyimino or ar(lower)alkoxyimino which may have one or more (preferably 1 to 3) higher alkoxy;

50 heterocyclithio(lower)alkanoyl which may have one or more (preferably 1 to 3) higher alkyl;

heterocyclic(lower)alkanoyl which may have one or more (preferably 1 to 3) lower alkoxyimino, higher alkyl, amino or protected amino;

ar(lower)alkoxyimino(lower)alkanoyl which may have one or more (preferably 1 to 3) higher alkoxy; higher alkanoyl;

55 ar(lower)alkenoyl which may have one or more (preferably 1 to 3) higher alkoxy;

higher alkenoyl; lower alkoxy carbonyl; higher alkoxy carbonyl; aryloxycarbonyl;

arylsulfonyl which may have one or more (preferably 1 to 3) lower alkyl or higher alkoxy;

aroyl which may have one or more (preferably 1 to 5) halogen, lower alkyl, higher alkyl, carboxy, lower

alkoxy which may have one or more (preferably 1 to 10) halogen, lower alkoxy(lower)alkoxy, ar(lower)alkoxy, higher alkoxy which may have one or more (preferably 1 to 17) halogen, higher alkenyloxy, aryl which may have one or more (preferably 1 to 3) higher alkoxy or aryloxy which may have one or more (preferably 1 to 3) lower alkoxy or higher alkoxy;

- 5 in which the more preferred one may be lower alkanoyl; halo(lower)alkanoyl; phenyl(lower)alkanoyl or naphthyl(lower)alkanoyl, each of which may have 1 to 3 hydroxy, lower alkoxy, higher alkoxy, phenyl, amino, lower alkoxycarbonylamino, di(lower)alkylamino, lower alkoxyimino, or phenyl-(lower)alkoxyimino which may have 1 to 3 higher alkoxy; pyridylthio(lower)alkanoyl which may have 1 to 3 higher alkyl;
- 10 imidazolyl(lower)alkanoyl or thiazolyl(lower)alkanoyl, each of which may have 1 to 3 lower alkoxyimino, higher alkyl, amino or lower alkoxycarbonylamino; phenyl(lower)alkoxyimino(lower)alkanoyl which may have 1 to 3 higher alkoxy; higher alkanoyl; phenyl(lower)alkenoyl which may have 1 to 3 higher alkoxy;
- 15 higher alkenoyl; lower alkoxycarbonyl, higher alkoxycarbonyl; phenoxycarbonyl; phenylsulfonyl or naphthylsulfonyl, each of which may have 1 to 3 lower alkyl or higher alkoxy; benzoyl, naphthoyl or anthrylcarbonyl, each of which may have 1 to 5 halogen, lower alkyl, higher alkyl, carboxy, lower alkoxy which may have 6 to 10 halogen, lower alkoxy(lower)alkoxy, phenyl(lower)alkoxy, higher alkoxy which may have 12 to 17 halogen, higher alkenyloxy, phenyl which may have 1 to 3 higher
- 20 alkoxy, phenoxy which may have 1 to 3 lower alkoxy or higher alkoxy; the much more preferred one may be (C<sub>1</sub>-C<sub>4</sub>)alkanoyl; halo(C<sub>1</sub>-C<sub>4</sub>)alkanoyl; phenyl(C<sub>1</sub>-C<sub>4</sub>)alkanoyl which may have 1 to 3 hydroxy, (C<sub>1</sub>-C<sub>4</sub>)alkoxy, (C<sub>7</sub>-C<sub>16</sub>)alkoxy, phenyl, amino, (C<sub>1</sub>-C<sub>4</sub>)alkoxycarbonylamino, di(C<sub>1</sub>-C<sub>4</sub>)alkylamino, (C<sub>1</sub>-C<sub>4</sub>)alkoxyimino or phenyl(C<sub>1</sub>-C<sub>4</sub>)alkoxyimino which may have (C<sub>7</sub>-C<sub>16</sub>)alkoxy;
- 25 naphthyl(C<sub>1</sub>-C<sub>4</sub>)alkanoyl which may have 1 to 3 (C<sub>1</sub>-C<sub>4</sub>)alkoxycarbonylamino; 1-(C<sub>7</sub>-C<sub>16</sub>)alkylpyridiniothio(C<sub>1</sub>-C<sub>4</sub>)alkanoyl; imidazolyl(C<sub>1</sub>-C<sub>4</sub>)alkanoyl which may have 1 to 3 (C<sub>7</sub>-C<sub>16</sub>)alkyl or (C<sub>1</sub>-C<sub>4</sub>)alkoxycarbonylamino; thiazolyl(C<sub>1</sub>-C<sub>4</sub>)alkanoyl which may have 1 to 3 (C<sub>1</sub>-C<sub>4</sub>)alkoxyimino or amino; phenyl(C<sub>1</sub>-C<sub>4</sub>)alkoxyimino(C<sub>1</sub>-C<sub>4</sub>)alkanoyl which may have 1 to 3 (C<sub>7</sub>-C<sub>16</sub>)alkoxy;
- 30 (C<sub>1</sub>-C<sub>17</sub>)alkyl; phenyl(C<sub>1</sub>-C<sub>4</sub>)alkenoyl which may have 1 to 3 (C<sub>7</sub>-C<sub>16</sub>)alkoxy; (C<sub>7</sub>-C<sub>18</sub>)alkenoyl; (C<sub>3</sub>-C<sub>6</sub>)alkoxycarbonyl; (C<sub>7</sub>-C<sub>16</sub>)alkoxycarbonyl; phenoxycarbonyl; phenylsulfonyl which may have (C<sub>1</sub>-C<sub>4</sub>)alkyl or (C<sub>7</sub>-C<sub>16</sub>)alkoxy; naphthylsulfonyl which may have (C<sub>7</sub>-C<sub>16</sub>)alkoxy;
- 35 benzoyl which may have 1 to 5 halogen, (C<sub>3</sub>-C<sub>6</sub>)alkyl, (C<sub>7</sub>-C<sub>16</sub>)alkyl, carboxy, (C<sub>1</sub>-C<sub>6</sub>)alkoxy which may have 6 to 10 halogen, (C<sub>1</sub>-C<sub>4</sub>)alkoxy(C<sub>1</sub>-C<sub>4</sub>)alkoxy, phenyl(C<sub>3</sub>-C<sub>6</sub>)alkoxy, (C<sub>7</sub>-C<sub>16</sub>)alkoxy which may have 12 to 17 halogen, phenyl which may have 1 to 3 (C<sub>7</sub>-C<sub>16</sub>)alkoxy or phenoxy which may have 1 to 3 (C<sub>3</sub>-C<sub>6</sub>)alkoxy or (C<sub>7</sub>-C<sub>16</sub>)alkoxy; naphthoyl which may have 1 to 3 (C<sub>3</sub>-C<sub>6</sub>)alkoxy (C<sub>7</sub>-C<sub>16</sub>)alkoxy or (C<sub>7</sub>-C<sub>16</sub>)alkenyloxy;
- 40 anthrylcarbonyl; and the most preferred one may be acetyl, 2-bromoacetyl, 2-(4-biphenyl)acetyl, 2-(4-octyloxyphenyl)acetyl, 3-(4-octyloxyphenyl)propionyl, 2-amino-2-(4-octyloxyphenyl)acetyl, 2-(t-butoxycarbonylamino)-2-(4-octyloxyphenyl)acetyl, 2-amino-3-(4-octyloxyphenyl)propionyl, 2-(t-butoxycarbonylamino)-3-(4-octyloxyphenyl)propionyl, 2-dimethylamino-3-(4-octyloxyphenyl)propionyl, 2-(t-butoxycarbonylamino)-2-(2-naphthyl)acetyl, 2-methoxy-2-(4-octyloxyphenyl)acetyl, 2-methoxyimino-2-(4-octyloxyphenyl)acetyl, 2-(4-octyloxybenzyloxyimino)-2-(4-hydroxyphenyl)acetyl, 2-(4-octyloxybenzyloxyimino)-2-phenylacetyl, 2-(4-octyloxybenzyloxyimino)acetyl, 2-(1-octyl-4-pyridinio)thioacetyl, 2-methoxyimino-2-(2-aminothiazol-4-yl)acetyl, 2-(t-butoxycarbonylamino)-3-(1-octyl-4-imidazolyl)propionyl, 3-(4-octyloxyphenyl)acryloyl, 3,7,11-trimethyl-2,6,10-dodecatrienoyl, t-butoxycarbonyl, octyloxycarbonyl, phenoxycarbonyl, p-tolylsulfonyl, 4-octyloxyphenylsulfonyl, 6-octyloxy-2-naphthylsulfonyl, 4-(t-butyl)benzoyl, 4-octylbenzoyl, 2,3,5,6-tetrafluoro-4-(2,2,3,3,4,4,5,5-octafluoropentyloxy)benzoyl, 4-(2-butoxyethoxy)benzoyl, 4-(4-phenylbutoxy)benzoyl, 4-octyloxybenzoyl, 2-carboxy-4-octyloxybenzoyl, 3-methoxy-4-octyloxybenzoyl, 4-(2,2,3,3,4,4,5,5,6,6,7,7,8,8-pentadecafluorooctyloxy)-2,3,5,6-tetrafluorobenzoyl, 4-(4-octyloxyphenyl)benzoyl, 4-(4-octyloxyphenoxy)benzoyl, 6-butoxy-2-naphthoyl, 6-hexyloxy-2-naphthoyl, 6-octyloxy-2-naphthoyl, 6-(2-ethylhexyloxy)-2-naphthoyl, 6-decyloxy-2-naphthoyl, 6-(3,7-dimethyloctyloxy)-2-naphthoyl, 6-dodecyloxy-2-naphthoyl, 6-(3,7-dimethyl-6-octenyloxy)-2-naphthoyl, 6-(3,7-dimethyl-2,6-octadienyloxy)-2-naphthoyl, 2-anthrylcarbonyl, 4-(4-heptyloxyphenyl)-benzoyl and 4-(4-hexyloxyphenoxy)benzoyl.

Suitable "acyl group exclusive of palmitoyl" can be referred to the ones is exemplified before for "acyl

group" except palmitoyl.

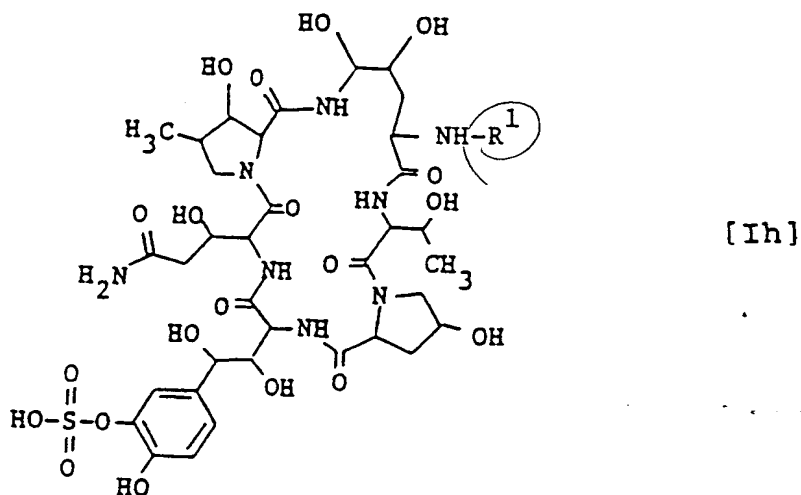
Suitable "ar(lower)alkanoyl" moiety in "ar(lower)alkanoyl which has higher alkoxy and protected amino" and "ar(lower)alkanoyl which has higher alkoxy and amino" can be referred to the ones as exemplified before for "acyl group" and suitable examples of the substituent(s) "higher alkoxy" and "protected amino" can be referred to the ones as exemplified before for "acyl group".

Suitable "halo(lower)alkanoyl" can be referred to the ones as exemplified before for "acyl group".

Suitable "pyridylthio(lower)alkanoyl" in "pyridylthio(lower)alkanoyl which may have higher alkyl" can be referred to the ones as exemplified before for "acyl group", and suitable examples of the substituent "higher alkyl" can be exemplified before for "acyl group".

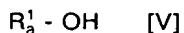
Suitable "acyloxy" may include hydroxysulfonyloxy, phosphonoxy, and the like.

In the object compound [I] thus defined, the following compound [Ih] is especially preferable.



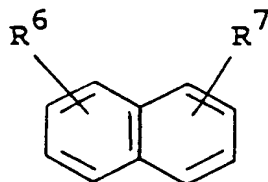
wherein  $R^1$  is hydrogen or acyl group,  
with proviso that  $R^1$  is not palmitoyl.

Suitable "acylating agent" for the acylation reaction is Process 2 may be an acid compound corresponding to the acyl group to be introduced or its reactive derivative at the carboxy group or a salt thereof and suitable example of said acylating agent is represented by the formula :



wherein  $R_a^1$  is as defined above,  
or its reactive derivative at the carboxy group or a salt thereof.

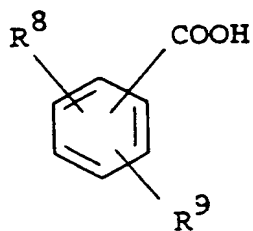
In the compound [V], the following compounds are novel.



[V-1]

or its reactive derivative  
at the carboxy group  
or a salt thereof

(2)



[V-2]

or its reactive derivative  
at the carboxy group  
or a salt thereof

wherein

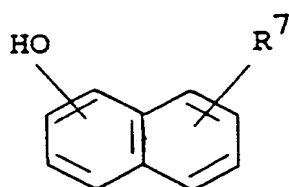
$R^6$  is lower alkoxy, higher alkoxy or higher alkenyloxy,

$R^7$  is  $-\text{COOH}$  or  $-\text{SO}_3\text{H}$ ,

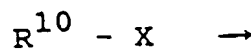
$R^8$  is 1 to 4 halogen,

$R^9$  is lower alkoxy which has one or more halogen, higher alkoxy which has one or more halogen.

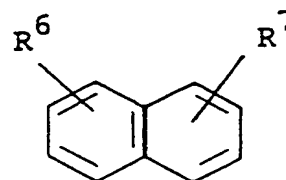
The compounds [V-1] and [V-2] can be prepared by the following processes.

Process B

+



→



[VI]

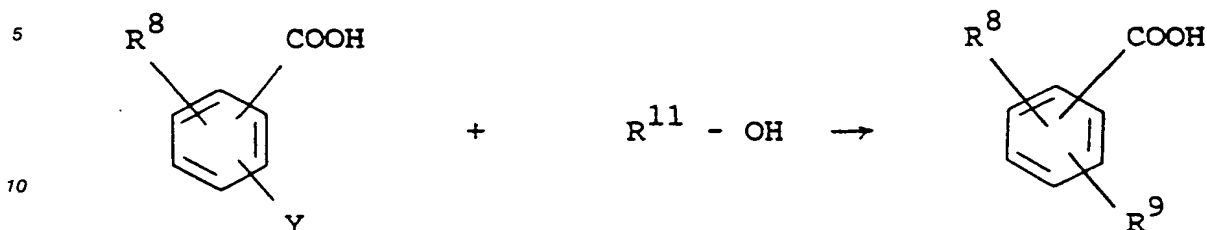
[VII]

[V-1]

or a salt thereof

or a salt thereof

or a salt thereof

Process C

[VIII]

[IX]

[V-2]

or a salt thereof

or a salt thereof

or a salt thereof

20 wherein

R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup> and R<sup>9</sup> are each as defined above,R<sup>10</sup> is lower alkyl, higher alkyl or higher alkenyl,R<sup>11</sup> is lower alkyl which has one or more halogen or higher alkyl which has one or more halogen, and

25 X and Y are each a leaving group.

In the above definitions, suitable "lower alkoxy", "higher alkoxy", "higher alkenyloxy", "halogen", "lower alkyl" and "higher alkyl" can be referred to the ones as exemplified before.

Suitable "higher alkenyl" may include 3-heptenyl, 7-octenyl, 2,6-octadienyl, 5-nonenyl, 1-decenyl, 3,7-dimethyl-6-octenyl, 3,7-dimethyl-2,6-octadienyl, 8-undecenyl, 3,6,8-dodecatrienyl, 5-tridecenyl, 7-tetradecenyl, 1,8-pentadecadienyl, 15-hexadecenyl, 11-heptadecenyl, 7-octadecenyl, 10-nonadecenyl, 18-icosenyl and the like, in which the preferred one may be (C<sub>7</sub>-C<sub>16</sub>)alkenyl.

As for R<sup>9</sup>, "lower alkoxy" has one or more (preferably 1 to 10, more preferably 6 to 10) halogen, and "higher alkoxy" has one or more (preferably 1 to 17, more preferably 12 to 17) halogen.

As for R<sup>11</sup>, "lower alkyl" has one or more (preferably 1 to 10, more preferably 6 to 10) halogen, and 35 "higher alkyl" has one or more (preferably 1 to 17, more preferably 12 to 17) halogen.

As for R<sup>6</sup>, preferred "lower alkoxy" may be (C<sub>4</sub>-C<sub>6</sub>)alkoxy.

Suitable "a leaving group" may include aforesaid halogen, lower alkanoyloxy (e.g. acetoxy, etc.), sulfonyloxy (e.g. mesyloxy, tosyloxy, etc.), and the like.

Regarding suitable salts and the reactive derivatives at the carboxy group of the compounds [V-1] and 40 [V-2], they can be referred to the ones as exemplified below for the compound [V].

The reactions in Processes B and C can be carried out according to the methods disclosed later in Preparations of the present specification or the similar manners thereto.

In the compound [V], there are other novel compounds than compounds [V-1] and [V-2], and they can be prepared, for example, by the methods disclosed later in Preparations.

45 Suitable "pyridinethione" in Process 4 may include 1,2-dihydropyridine-2-thione, 1,4-dihydropyridine-4-thione, and the like, and said "pyridinethione" may have aforesaid "higher alkyl".

The processes for preparing the object compound [I] or a salt thereof of the present invention are explained in detail in the following.

50 Process 1

The object compound [Ia] or a salt thereof can be prepared by subjecting a compound [II] or a salt thereof to elimination reaction of N-acyl group.

This reaction is carried out in accordance with a conventional method such as hydrolysis, reduction, 55 reaction with an enzyme or the like.

The hydrolysis is preferably carried out in the presence of a base or an acid including Lewis acid. Suitable base may include an inorganic base and an organic base such as an alkali metal [e.g. sodium, potassium, etc.], an alkaline earth metal [e.g. magnesium, calcium, etc.], the hydroxide or carbonate or

bicarbonate thereof, trialkylamine [e.g. trimethylamine, triethylamine, etc.], picoline, 1,5-diazabicyclo[4.3.0]-non-5-ene, 1,4-diazabicyclo[2.2.2]octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, or the like.

Suitable acid may include an organic acid [e.g. formic acid, acetic acid, propionic acid, trichloroacetic acid, trifluoroacetic acid, etc.] and an inorganic acid [e.g. hydrochloric acid, hydrobromic acid, sulfuric acid, hydrogen chloride, hydrogen bromide, etc.]. The elimination using Lewis acid such as trihaloacetic acid [e.g. trichloroacetic acid, trifluoroacetic acid, etc.] or the like is preferably carried out in the presence of cation trapping agents [e.g. anisole, phenol, etc.].

The reaction is usually carried out in a solvent such as water, an alcohol [e.g. methanol, ethanol, etc.], methylene chloride, tetrahydrofuran, a mixture thereof or any other solvent which does not adversely influence the reaction. A liquid base or acid can be also used as the solvent. The reaction temperature is not critical and the reaction is usually carried out under cooling to warming.

The reduction method applicable for the elimination reaction may include chemical reduction and catalytic reduction.

Suitable reducing agents to be used in chemical reduction are a combination of metal [e.g. tin, zinc, iron, etc.] or metallic compound [e.g. chromium chloride, chromium acetate, etc.] and an organic or inorganic acid [e.g. formic acid, acetic acid, propionic acid, trifluoroacetic acid, p-toluenesulfonic acid, hydrochloric acid, hydrobromic acid, etc.].

Suitable catalysts to be used in catalytic reduction are conventional ones such as platinum catalysts [e.g. platinum plate, spongy platinum, platinum black, colloidal platinum, platinum oxide, platinum wire, etc.], palladium catalysts [e.g. spongy palladium, palladium black, palladium oxide, palladium on carbon, colloidal palladium, palladium on barium sulfate, palladium on barium carbonate, etc.], nickel catalysts [e.g. reduced nickel, nickel oxide, Raney nickel, etc.], cobalt catalysts [e.g. reduced cobalt, Raney cobalt, etc.], iron catalysts [e.g. reduced iron, Raney iron, etc.], copper catalysts [e.g. reduced copper, Raney copper, Ullman copper, etc.] and the like.

The reduction is usually carried out in a conventional solvent which does not adversely influence the reaction such as water, methanol, ethanol, propanol, N,N-dimethylformamide, or a mixture thereof. Additionally, in case that the above-mentioned acids to be used in chemical reduction are in liquid, they can also be used as a solvent. Further, a suitable solvent to be used in catalytic reduction may be the above-mentioned solvent, and other conventional solvent such as diethyl ether, dioxane, tetrahydrofuran, etc., or a mixture thereof.

The reaction temperature of this reduction is not critical and the reaction is usually carried out under cooling to warming.

The reaction with an enzyme can be carried out by reacting the compound [II] or a salt thereof with an enzyme suitable for the elimination reaction of N-acyl group.

Suitable example of said enzyme may include the one produced by certain microorganisms of the Actinoplanaceae, for example, *Actinoplanes utahensis* IFO-13244, *Actinoplanes utahensis* ATCC 12301, *Actinoplanes missouriensis* NRRL 12053, or the like; and the like.

This elimination reaction is usually carried out in a solvent such as phosphate buffer, Tris-HCl buffer or any other solvent which does not adversely influence the reaction.

The reaction temperature is not critical and the reaction can be carried out at room temperature or under warming.

## Process 2

The object compound [Ib] or a salt thereof can be prepared by subjecting the compound [Ia] or a salt thereof to acylation reaction.

The acylation reaction of this process can be carried out by reacting the compound [Ia] or a salt thereof with aforesaid "acylating agent", for example, the compound [V] or its reactive derivative at the carboxy group or a salt thereof.

Suitable reactive derivative at the carboxy group of the compound [V] may include an acid halide, an acid anhydride, an activated amide, an activated ester, and the like. Suitable examples of the reactive derivatives may be an acid chloride; an acid azide; a mixed acid anhydride with an acid such as substituted phosphoric acid [e.g. dialkylphosphoric acid, phenylphosphoric acid, diphenylphosphoric acid, dibenzylphosphoric acid, halogenated phosphoric acid, etc.], dialkylphosphorous acid, sulfurous acid, thiosulfuric acid, sulfuric acid, sulfonic acid [e.g. methanesulfonic acid, etc.], aliphatic carboxylic acid [e.g. acetic acid, propionic acid, butyric acid, isobutyric acid, pivalic acid, pentanoic acid, isopentanoic acid, 2-ethylbutyric acid, trichloroacetic acid, etc.]; or aromatic carboxylic acid [e.g. benzoic acid, etc.]; a symmetrical acid anhydride; an activated amide with imidazole, 4-substituted imidazole, dimethylpyrazole, triazole, tetrazole

or 1-hydroxy-1H-benzotriazole; or an activated ester [e.g. cyanomethyl ester, methoxymethyl ester, dimethyliminomethyl  $[(CH_3)_2N=CH-]$  ester, vinyl ester, propargyl ester, p-nitrophenyl ester, 2,4-dinitrophenyl ester, trichlorophenyl ester, pentachlorophenyl ester, mesylphenyl ester, phenylazophenyl ester, phenyl thioester, p-nitrophenyl thioester, p-cresyl thioester, carboxymethyl thioester, pyranyl ester, pyridyl ester, piperidyl ester, 8-quinolyl thioester, etc.], or an ester with a N-hydroxy compound [e.g. N,N-dimethylhydroxylamine, 1-hydroxy-2-(1H)-pyridone, N-hydroxysuccinimide, N-hydroxyphthalimide, 1-hydroxy-1H-benzotriazole, etc.], and the like. These reactive derivatives can optionally be selected from them according to the kind of the compound [V] to be used.

Suitable salts of the compound [V] and its reactive derivative can be referred to the ones as exemplified for the compound [I].

The reaction is usually carried out in a conventional solvent such as water, alcohol [e.g. methanol, ethanol, etc.], acetone, dioxane, acetonitrile, chloroform, methylene chloride, ethylene chloride, tetrahydrofuran, ethyl acetate, N,N-dimethylformamide, pyridine or any other organic solvent which does not adversely influence the reaction. These conventional solvent may also be used in a mixture with water.

In this reaction, when the compound [V] is used in a free acid form or its salt form, the reaction is preferably carried out in the presence of a conventional condensing agent such as N,N'-dicyclohexylcarbodiimide; N-cyclohexyl-N'-morpholinoethylcarbodiimide; N-cyclohexyl-N'-(4-diethylaminocyclohexyl)-carbodiimide; N,N'-diethylcarbodiimide, N,N'-diisopropylcarbodiimide; N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide, N,N'-carbonylbis-(2-methylimidazole); pentamethyleneketene-N-cyclohexylimine; diphenylketene-N-cyclohexylimine; ethoxyacetylene; 1-alkoxy-1-chloroethylene; trialkyl phosphite; ethyl polyphosphate; isopropyl polyphosphate; phosphorus oxychloride (phosphoryl chloride); phosphorus trichloride; thionyl chloride; oxalyl chloride; lower alkyl haloformate [e.g. ethyl chloroformate, isopropyl chloroformate, etc.]; triphenylphosphine; 2-ethyl-7-hydroxybenzisoxazolium salt; 2-ethyl-5-(m-sulfophenyl)isoxazolium hydroxide intramolecular salt; 1-(p-chlorobenzenesulfonyloxy)-6-chloro-1H-benzotriazole; so-called Vilsmeier reagent prepared by the reaction of N,N-dimethylformamide with thionyl chloride, phosgene, trichloromethyl chloroformate, phosphorus oxychloride, methanesulfonyl chloride, etc.; or the like.

The reaction may also be carried out in the presence of an inorganic or organic base such as an alkali metal carbonate, alkali metal bicarbonate, tri(lower)alkylamine, pyridine, di(lower)alkylaminopyridine (e.g. 4-dimethylaminopyridine, etc.), N-(lower)alkylmorpholine, N,N-di(lower)alkylbenzylamine, or the like.

The reaction temperature is not critical, and the reaction is usually carried out under cooling to warming.

### Process 3

The object compound [Id] or a salt thereof can be prepared by subjecting a compound [Ic] or a salt thereof to elimination reaction of amino protective group.

Suitable salts of the compounds [Ic] and [Id] can be referred to the ones as exemplified for the compound [I].

This elimination reaction can be carried out in accordance with a conventional method as explained above for Process 1.

### Process 4

The object compound [If] or a salt thereof can be prepared by reacting a compound [Ie] or a salt thereof with a compound [III] or a salt thereof.

Suitable salt of the compound [If] can be referred to the ones as exemplified for the compound [I].

Suitable salt of the compound [III] can be referred to acid addition salts as exemplified for the compound [I].

The present reaction may be carried out in a solvent such as water, phosphate buffer, acetone, chloroform, acetonitrile, nitrobenzene, methylene chloride, ethylene chloride, formamide, N,N-dimethylformamide, methanol, ethanol, diethyl ether, tetrahydrofuran, dimethyl sulfoxide, or any other organic solvent which does not adversely affect the reaction, preferably in ones having strong polarities. Among the solvents, hydrophilic solvents may be used in a mixture with water. When the compound [III] is in liquid, it can also be used as a solvent.

The reaction is preferably conducted in the presence of a base, for example, inorganic base such as alkali metal hydroxide, alkali metal carbonate, alkali metal bicarbonate, organic base such as trialkylamine, and the like.

The reaction temperature is not critical, and the reaction is usually carried out under cooling, at room temperature, under warming or under heating.

The present reaction is preferably carried out in the presence of alkali metal halide [e.g. sodium iodide, potassium iodide, etc.], alkali metal thiocyanate [e.g. sodium thiocyanate, potassium thiocyanate, etc.] or the like.

## 5 Process 5

The object compound [Ig] or a salt thereof can be prepared by subjecting a compound [IV] or a salt thereof to acylation reaction.

Suitable salts of the compounds [Ig] and [IV] can be referred to the ones as exemplified for the  
10 compound [I].

Suitable "acylating agent" in this Process 5 may be an acid compound corresponding to the acyl group to be introduced, for example, phosphoric acid and its derivative (e.g. phosphoryl chloride, diphenylphosphorochloridate, etc.), sulfuric acid and its derivative [e.g. sulfur trioxide-pyridine, sulfur trioxide-tri-(lower)alkylamine (e.g. trimethylamine, triethylamine, etc.), chlorosulfonic acid, etc.], or the like.

15 This reaction can be carried out in a conventional manner.

The process for preparing the starting compound [II] or a salt thereof of the present invention is explained in detail in the following.

## Process A

20 The compound [II] or a salt thereof can be prepared by the fermentation process.

The fermentation process is explained in detail in the following.

The compound [II] or a salt thereof of this invention can be produced by fermentation of the compound [II] or a salt thereof-producing strain belonging to the genus Coleophoma such as Coleophoma sp. F-11899  
25 in a nutrient medium.

### (i) Microorganism :

Particulars of the microorganism used for producing the compound [II] or a salt thereof is explained in  
30 the following.

The strain F-11899 was originally isolated from a soil sample collected at Iwaki-shi, Fukushima-ken, Japan. This organism grew rather restrictedly on various culture media, and formed dark grey to brownish grey colonies. Anamorph (conidiomata) produced on a steam-sterilized leaf segment affixed on a Miura's LCA plate <sup>1)</sup> or a corn meal agar plate by inoculating the isolate, while neither teleomorph nor anamorph  
35 formed on the agar media. Its morphological, cultural and physiological characteristics are as follows.

Cultural characteristics on various agar media are summarized in Table 1. Cultures on potato dextrose agar grew rather rapidly, attaining 3.5-4.0 cm in diameter after two weeks at 25 °C. This colony surface was plane, felty, somewhat wrinkly and brownish grey. The colony center was pale grey to brownish grey, and covered with aerial hyphae. The reverse color was dark grey. Colonies on malt extract agar grew more  
40 restrictedly, attaining 2.5-3.0 cm in diameter under the same conditions. The surface was plane, thin to felty and olive brown. The colony center was yellowish grey, and covered with aerial hyphae. The reverse was brownish grey.

The morphological characteristics were determined on basis of the cultures on a sterilized leaf affixed to a Miura's LCA plate. Conidiomata formed on the leaf segment alone. They were pycnidial, superficial,  
45 separate, discoid to ampulliform, flattened at the base, unilocular, thin-walled, black, 90-160(-200) µm in diameter and 40-70 µm high. Ostiole was often single, circular, central, papillate, 10-30 µm in diameter and 10-20 µm high. Conidiophores formed from the lower layer of inner pycnidial walls. They were hyaline, simple or sparingly branched, septate and smooth. Conidiogenous cells were enteroblastic, phialidic, determinate, ampulliform to obpyriform, hyaline, smooth, 5-8 x 4-6 µm, with a collarette. The collarettes  
50 were campanulate to cylindrical, and 14-18 x 3-5 µm. Conidia were hyaline, cylindrical, thin-walled, aseptate, smooth and 14-16(-18) x 2-3 µm.

The vegetative hyphae were septate, brown, smooth and branched. The hyphal cells were cylindrical and 2-7 µm thick. The chlamydospores were absent.

The strain F-11899 had a temperature range for growth of 0 to 31 °C and an optimum temperature of 23  
55 to 27 °C on potato dextrose agar.

1) Miura, K. and M. Y. Kudo: An agar-medium for aquatic Hyphomycetes., Trans. Ycolo. Soc. Japan, 11:116-118, 1970.



The above characteristics indicate that the strain F-11899 belongs to the order Coelomycetes <sup>2), 3), 4)</sup>. Thus, we named the strain "Coelomycetes strain F-11899".

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2) Arx, J. A. von: The Genera of Fungi - Sportulating in Pure Culture (3rd ed.), 315 p., J. Cramer, Vaduz, 1974.

3) Sutton. B. C.: The Coelomycetes - Fungi Imperfecti with Pycnidia, Acervuli and Stromata., 696 p., Commonwealth Mycological Institute, Kew, 1980.

4) Hawksworth, D. L., B. C. Sutton and G. C. Ainsworth: Dictionary of the Fungi (7th ed.), 445 p., Commonwealth Mycological Institute, Kew., 1983.

Table 1 Cultural characteristics of the strain F-11899

Medium	Cultural characteristics
Malt extract agar (Blakeslee 1915)	G: Rather restrictedly, 2.5-3.0 cm S: Circular, plane, thin to felty, olive brown (4F5), arising aerial hyphae at the center (yellowish grey (4B2)) R: Brownish grey (4F2)
Potato dextrose agar (Difco 0013)	G: Rather rapidly, 3.5-4.0 cm S: Circular, plane, felty, somewhat wrinkly, brownish grey (4F2), arising aerial hyphae at the center (pale grey (4B1) to brownish grey (4F2)) R: Dark grey (4F1)
Czapeck's solution agar (Raper and Thom 1949)	G: Very restrictedly, 1.0-1.5 cm S: Irregular, thin, scanty, immersed, subhyaline to white R: Subhyaline to white
Sabouraud dextrose agar (Difco 0109)	G: Restrictedly, 2.0-2.5 cm S: Circular, plane, thin, white, sectoring, light brown (6D5) at the colony center R: Pale yellow (4A3)
Oatmeal agar (Difco 0552)	G: Fairly rapidly, 4.0-4.5 cm S: Circular, plane, felty to cottony, dark grey (4F1) to brownish grey (4F2) R: Brownish grey (4D2)

nitrogen base dextrose medium. To a 50  $\mu$ l sample solution with serial 2-fold dilution was added a 50  $\mu$ l of microorganism suspension in saline to yield a final concentration of  $1 \times 10^5$  colony forming units/ml. The Candida cultures were incubated at 37 °C for 22 hours. After incubation, the growth of microorganism in each well was determined by measuring the turbidity. The results were shown as IC<sub>50</sub> value in which concentration the turbidity was half of that in the well without sample. The results are shown in Table 2.

Table 2

organism	IC <sub>50</sub>
Candida albicans FP578	0.31
Candida tropicalis YC118	0.47

#### Test 2 Acute toxicity of FR131535 substance :

The acute toxicity of the FR131535 substance was determined to ICR mice (female, 4 weeks old) by a single intravenous injection. No toxic symptom was observed at the dose of 500 mg/kg.

#### Test 3 Antimicrobial activity (2) :

In vitro antimicrobial activity of the compound of Example 12 disclosed later (hereinafter referred to as FR139687 substance) was determined by the two-fold agar-plate dilution method as described below.

One loopful of an overnight culture of each test microorganism in Sabouraud broth containing 2 % Glucose ( $10^5$  viable cells per ml) was streaked on yeast nitrogen base dextrose agar (YNBDA) containing graded concentrations of the FR139687 substance, and the minimal inhibitory concentration (MIC) was expressed in terms of  $\mu$ g/ml after incubation at 30 °C for 24 hours.

organism	MIC ( $\mu$ g/ml)
Candida albicans YU-1200	0.05

From the test results, it is realized that the polypeptide compound [I] of the present invention has an anti-microbial activity (especially, antifungal activity).

The pharmaceutical composition of this invention can be used in the form of a pharmaceutical preparation, for example, in solid, semisolid or liquid form, which contains the polypeptide compound [I] or a pharmaceutically acceptable salt thereof, as an active ingredient in admixture with an organic or inorganic carrier or excipient suitable for rectal, pulmonary (nasal or buccal inhalation), nasal, ocular, external (topical), oral or parenteral (including subcutaneous, intravenous and intramuscular) administrations or insufflation. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, pellets, troches, capsules, suppositories, creams, ointments, aerosols, powders for insufflation, solutions, emulsions, suspensions, and any other form suitable for use. And, if necessary, in addition, auxiliary, stabilizing, thickening and coloring agents and perfumes may be used. The polypeptide compound [I] or a pharmaceutical acceptable salt thereof is/are included in the pharmaceutical composition in an amount sufficient to produce the desired antimicrobial effect upon the process or condition of diseases.

For applying the composition to human, it is preferable to apply it by intravenous, intramuscular, pulmonary, or oral administration, or insufflation. While the dosage of therapeutically effective amount of the polypeptide compound [I] varies from and also depends upon the age and condition of each individual patient to be treated, in the case of intravenous administration, a daily dose of 0.01 - 20 mg of the polypeptide compound [I] per kg weight of human being, in the case of intramuscular administration, a daily dose of 0.1 - 20 mg of the polypeptide compound [I] per kg weight of human being, in case of oral administration, a daily dose of 0.5 - 50 mg of the polypeptide compound [I] per kg weight of human being is generally given for treating or preventing infectious diseases.

The following Preparations and Examples are given for the purpose of illustrating the present invention in more detail.

Preparation 1

To methanol (50 ml) was added thionyl chloride (8.73 ml) at  $-5^{\circ}\text{C}$  and the mixture was stirred for 10 minutes and then D-2-(p-hydroxyphenyl)glycine (5 g) was added thereto under ice-cooling. The mixture was stirred for 12 hours at room temperature. The reaction mixture was evaporated under reduced pressure to give D-2-(p-hydroxyphenyl)glycine methyl ester hydrochloride (6.3 g).

IR (Nujol) : 3380, 1720, 1580, 1250  $\text{cm}^{-1}$

NMR ( $\text{DMSO}-d_6$ ,  $\delta$ ) : 3.70 (3H, s), 5.11 (1H, s), 6.83 (2H, d,  $J=8.6\text{Hz}$ ), 7.28 (2H, d,  $J=8.6\text{Hz}$ ), 8.91 (2H s), 9.93 (1H, s)

Preparation 2

To a solution of D-2-(p-hydroxyphenyl)glycine methyl ester hydrochloride (6.3 g) and triethylamine (8.71 ml) in tetrahydrofuran (100 ml) was added di-t-butyl dicarbonate (6.82 g). The mixture was stirred for 2 hours at room temperature. The reaction mixture was added to diethyl ether (1 l) and an insoluble material was filtered off, and the filtrate was evaporated under reduced pressure to give N-(t-butoxycarbonyl)-D-2-(p-hydroxyphenyl)glycine methyl ester (6.83 g).

IR (Nujol) : 3420, 3350, 1720, 1660  $\text{cm}^{-1}$

NMR ( $\text{DMSO}-d_6$ ,  $\delta$ ) : 1.38 (9H, s), 3.59 (3H, s), 5.05 (1H, d,  $J=7.9\text{Hz}$ ), 6.70 (2H, d,  $J=8.5\text{Hz}$ ), 7.16 (2H, d,  $J=8.5\text{Hz}$ ), 7.60 (1H, d,  $J=7.9\text{Hz}$ ), 9.48 (1H, s)

Preparation 3

To a suspension of N-(t-butoxycarbonyl)-D-2-(p-hydroxyphenyl)glycine methyl ester (6.8 g) and potassium bicarbonate (1.84 g) in N,N-dimethylformamide (34 ml) was added octyl bromide (4.176 ml). The mixture was stirred for 6 hours at  $60^{\circ}\text{C}$ . The reaction mixture was added to a mixture of water and ethyl acetate. The organic layer was separated and dried over magnesium sulfate. The magnesium sulfate was filtered off, and the filtrate was evaporated under reduced pressure to give N-(t-butoxycarbonyl)-D-2-(p-octyloxyphenyl)glycine methyl ester (6.96 g).

IR (Nujol) : 1710, 1490, 1240, 1160  $\text{cm}^{-1}$

NMR ( $\text{DMSO}-d_6$ ,  $\delta$ ) : 0.859 (3H, t,  $J=6.2\text{Hz}$ ), 1.17-1.33 (10H, m), 1.38 (9H, s), 1.60-1.80 (2H, m), 3.59 (3H, s), 3.93 (2H, t,  $J=6.3\text{Hz}$ ), 5.11 (1H, d,  $J=7.9\text{Hz}$ ), 6.87 (2H, d,  $J=8.7\text{Hz}$ ), 7.27 (2H, d,  $J=8.7\text{Hz}$ ), 7.68 (1H, d,  $J=7.9\text{Hz}$ )

Preparation 4

To 4N aqueous solution of sodium hydroxide (8.77 ml) was added N-(t-butoxycarbonyl)-D-2-(p-octyloxyphenyl)glycine methyl ester (6.9 g) and stirred for 1.5 hours at room temperature. The reaction mixture was added to a mixture of water and ethyl acetate and 1N hydrochloric acid was added thereto to adjust the mixture to pH 3. The organic layer was separated and dried over magnesium sulfate. The magnesium sulfate was filtered off, and the filtrate was evaporated under reduced pressure to give N-(t-butoxycarbonyl)-D-2-(p-octyloxyphenyl)glycine (3.9 g).

NMR ( $\text{DMSO}-d_6$ ,  $\delta$ ) : 0.860 (3H, t,  $J=6.8\text{Hz}$ ), 1.17-1.33 (10H, m), 1.38 (9H, s), 1.60-1.80 (2H, m), 3.93 (2H, t,  $J=6.4\text{Hz}$ ), 5.10 (1H, d,  $J=8.2\text{Hz}$ ), 6.87 (2H, d,  $J=8.7\text{Hz}$ ), 7.28 (2H, d,  $J=8.7\text{Hz}$ ), 7.46 (1H, d,  $J=8.2\text{Hz}$ )

Preparation 5

To a solution of N-(t-butoxycarbonyl)-D-2-(p-octyloxyphenyl)glycine (1 g) in acetonitrile (10 ml) and pyridine (0.213 ml) in acetonitrile (10 ml) was added N,N'-disuccinimidyl carbonate (0.675 g). The mixture was stirred for 12 hours at room temperature. The reaction mixture was added to a mixture of water and ethyl acetate. The organic layer was separated and dried over magnesium sulfate. The magnesium sulfate was filtered off, and the filtrate was evaporated under reduced pressure to give N-(t-butoxycarbonyl)-D-2-(p-octyloxyphenyl)glycine succinimido ester (0.92 g).

IR (Nujol) : 3350, 1810, 1730, 1680  $\text{cm}^{-1}$

NMR ( $\text{DMSO}-d_6$ ,  $\delta$ ) : 0.862 (3H, t,  $J=6.7\text{Hz}$ ), 1.17-1.33 (10H, m), 1.40 (9H, s), 1.60-1.80 (2H, m), 2.77 (4H, s), 3.97 (2H, t,  $J=6.5\text{Hz}$ ), 5.54 (1H, d,  $J=8.1\text{Hz}$ ), 6.91 (2H, d,  $J=8.7\text{Hz}$ ), 7.39 (2H, d,  $J=8.7\text{Hz}$ ), 8.05 (1H, d,  $J=8.1\text{Hz}$ )

Preparation 6

N-(t-Butoxycarbonyl)-L-tyrosine methyl ester was obtained according to a similar manner to that of

5 Preparation 2.

IR (Nujol) : 3430, 3360, 1730, 1670, 1170  $\text{cm}^{-1}$   
 NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.33 (9H, s), 2.90 (2H, m), 3.59 (3H, s), 4.05 (1H, m), 6.65 (2H, d,  $J=8.4\text{Hz}$ ),  
 7.00 (2H, d,  $J=8.4\text{Hz}$ ), 7.21 (1H, d,  $J=8.0\text{Hz}$ ), 9.22 (1H, s)

10

Preparation 7

O<sup>4</sup>-Octyl-N-(t-butoxycarbonyl)-L-tyrosine methyl ester was obtained according to a similar manner to that of

15

Preparation 3.

IR (Nujol) : 3350, 1735, 1685, 1250, 1170  $\text{cm}^{-1}$   
 NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.859 (3H, t,  $J=6.7\text{Hz}$ ), 1.20-1.30 (10H, m), 1.68 (2H, quintet,  $J=7.3\text{Hz}$ ), 2.82  
 (2H, m), 3.60 (3H, s), 3.91 (2H, t,  $J=7.3\text{Hz}$ ), 4.08 (1H, m), 6.81 (2H, d,  
 $J=8.6\text{Hz}$ ), 7.12 (2H, d,  $J=8.6\text{Hz}$ ), 7.25 (1H, d,  $J=8.0\text{Hz}$ )

20

Preparation 8

O<sup>4</sup>-Octyl-N-(t-butoxycarbonyl)-L-tyrosine was obtained according to a similar manner to that of Preparation 4.

25

IR (Nujol) : 3400-2900 (br), 1700, 1240, 1160  $\text{cm}^{-1}$   
 NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.859 (3H, t,  $J=6.8\text{Hz}$ ), 1.20-1.30 (10H, m), 1.32 (9H, s), 1.68 (2H, quintet,  
 $J=7.0\text{Hz}$ ), 2.67-2.95 (1H, m), 3.90 (2H, t,  $J=7.0\text{Hz}$ ), 4.01 (1H, m), 6.81 (2H, d,  
 $J=8.6\text{Hz}$ ), 7.02 (1H, d,  $J=8.3\text{Hz}$ ), 7.13 (2H, d,  $J=8.6\text{Hz}$ )

30

Preparation 9

O<sup>4</sup>-Octyl-N-(t-butoxycarbonyl)-L-tyrosine succinimido ester was obtained according to a similar manner to that of Preparation 5.

35

IR (Nujol) : 3350, 1780, 1720, 1690  $\text{cm}^{-1}$   
 NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.860 (3H, t,  $J=6.7\text{Hz}$ ), 1.20-1.30 (10H, m), 1.32 (9H, s), 1.68 (2H, quintet,  
 $J=7.0\text{Hz}$ ), 2.82 (4H, s), 2.80-3.20 (1H, m), 3.92 (2H, t,  $J=7.0\text{Hz}$ ), 4.44 (1H, m),  
 6.81 (2H, d,  $J=8.5\text{Hz}$ ), 7.22 (2H, d,  $J=8.5\text{Hz}$ ), 7.60 (1H, d,  $J=8.3\text{Hz}$ )

40

Preparation 10

(1) A seed medium (160 ml) consisting of sucrose 4%, cotton seed flour 2%, dried yeast 1%, peptone 1%,  $\text{KH}_2\text{PO}_4$  0.2%,  $\text{CaCO}_3$  0.2% and Tween 80 (made by NAKARAI CHEMICALS LTD.) 0.1% was poured into  
 each of two 500 ml Erlenmeyer flasks and sterilized at 121 °C for 30 minutes. A loopful of slant culture of  
*Coleophoma* sp. F-11899 was inoculated to each of the medium and cultured under shaking condition at  
 25 °C for 4 days.

45

A production medium (20 liters) consisting of Pine Dex #3 (made by Matsutani Chemical Ltd.) 3%, glucose 1%, wheat germ 1%, cotton seed flour 0.5%,  $\text{KH}_2\text{PO}_4$  2%,  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  1.5%,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$   
 0.001% and Adekanol (defoaming agent, made by Asahi Denka Co., Ltd.) 0.05% was poured into a 30 liter-  
 jar fermentor and sterilized at 121 °C for 30 minutes.

50

The resultant seed culture broth (320 ml) was inoculated to the production medium and cultured at  
 25 °C for 4 days, agitated at 200 rpm and aerated at 20 liters per minute. To the cultured broth thus  
 obtained (20 liters) was added an equal volume of acetone. After occasionally stirring at room temperature  
 for a while, the broth was filtered. The filtrate was concentrated in vacuo to remove acetone. The aqueous  
 filtrate (10 liters) was washed with two equal volume of ethyl acetate and extracted with n-butanol (10 liters)  
 twice. The combined n-butanol layer was concentrated in vacuo and the residue was applied on a column  
 (300 ml) of Silica gel 60 (made by E. Merck) and eluted with a stepwise organic solvent mixture consisting

55

of dichloromethane-methanol. The fractions having anti-Candida activity were eluted in the range of the solvent mixture (3:1 through 1:1). The active fractions were combined and concentrated in vacuo to dryness. The residue was dissolved in 50% aqueous methanol (15 ml) and applied on a column (250 ml) of ODS YMC GEL (made by Yamamura Chemical Lab.). The column was washed with 50% aqueous methanol and  
 5 eluted with 80% aqueous methanol. The eluate was concentrated and was further purified on a centrifugal partition chromatography (CPC) using a solvent system n-butanol:methanol:water (4:1:5) of upper stationary phase and lower mobile phase in a descending model. The pooled fractions containing the object compound (major component) were concentrated in vacuo and applied on a column (35 ml) of silica gel 60. The column was developed with n-butanol:acetic acid:water (6:1:1). The active fractions were combined and  
 10 concentrated in vacuo to dryness and dissolved in a small volume of 50% aqueous methanol. The solution was passed through a column (3.5 ml) of ODS YMC GEL. The column was washed with 50% aqueous methanol and eluted with methanol. The eluate was concentrated to dryness, dissolved in a small volume of water and adjusted to pH 7.0 with 0.01N NaOH. The solution was freeze-dried to give a white powder of said compound in its sodium salt form (hereinafter referred to as FR901379 substance) (11 mg).

15 The FR901379 substance as obtained has the following physico-chemical properties.

Appearance :

white powder

Nature :

neutral substance

20 Melting point :

215-221 °C (dec.)

Specific rotation :

$[\alpha]_D^{23}$  -20.3 (C: 0.5, H<sub>2</sub>O)

Molecular formula :

25 C<sub>51</sub>H<sub>81</sub>N<sub>8</sub>O<sub>21</sub>SNa

#### Elemental Analysis :

Calcd. : for C <sub>51</sub> H <sub>81</sub> N <sub>8</sub> SO <sub>21</sub> Na	C 51.17,	H 6.77,	N 9.36,	S 2.68 (%)
Found :	C 49.61,	H 7.58,	N 7.65,	S 2.14 (%)

35 Molecular weight :

HRFAB-MS : 1219.5078

(Calcd for C<sub>51</sub>H<sub>82</sub>N<sub>8</sub>SO<sub>21</sub> + 2Na - H: 1219.5032)

40 Solubility :

soluble : methanol, water

slightly soluble : ethyl acetate, acetone

insoluble : chloroform, n-hexane

45 Color reaction :

positive : iodine vapor reaction, cerium sulfate reaction, ferric chloride reaction, Ninhydrin reaction

negative : Dragendorff reaction, Ehrlich reaction

## Thin layer chromatography (TLC) :

Stationary phase	Developing solvent	Rf value
silica gel*	n-butanol:acetic acid; water (3:1:1)	0.36
	ethyl acetate:isopropyl alcohol:water (5:3:1)	0.31

\* Silica Gel 60 (made by E. Merck)

## Ultraviolet absorption spectrum :

$\lambda_{\text{max}}^{\text{methanol}} (E_{1\text{cm}}^{1\%})$  : 207(169), 276(13.5), 225(sh),  
283(sh) nm

$\lambda_{\text{max}}^{\text{methanol}+0.01\text{N-NaOH}} (E_{1\text{cm}}^{1\%})$  : 209(232), 244(59.5),  
284(13.5), 294(sh) nm

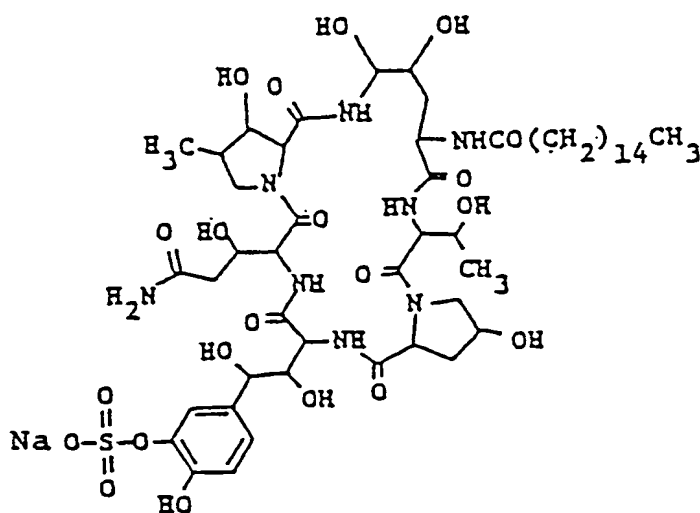
## Infrared absorption spectrum:

$\nu_{\text{max}}^{\text{KBr}}$  : 3350, 2920, 2840, 1660, 1625, 1530, 1510,  
1435, 1270, 1240, 1070, 1045, 800, 755,  
710  $\text{cm}^{-1}$

 $^1\text{H}$  Nuclear magnetic resonance spectrum :(CD<sub>3</sub>OD, 400MHz)

$\delta$  : 7.30 (1H d, J=2Hz), 7.03 (1H, dd, J=8 and 2Hz), 6.85 (1H, d, J=8Hz), 5.23 (1H, d, J=3Hz), 5.06  
(1H, d, J=4Hz), 4.93 (1H, d, J=3Hz), 4.59-4.51 (3H, m), 4.47-4.35 (5H, m), 4.29 (1H, dd, J=6 and  
2Hz), 4.17 (1H, m), 4.07 (1H, m), 3.95-3.89 (2H, m), 3.76 (1H, broad d, J=11Hz), 3.36 (1H, m),  
2.75 (1H, dd, J=16 and 4Hz), 2.50 (1H, m), 2.47 (1H, dd, J=16 and 9Hz), 2.38 (1H, m), 2.21 (2H,  
m), 2.03-1.93 (3H, m), 1.57 (2H, m), 1.45-1.20 (24H, m), 1.19 (3H, d, J=6Hz), 1.08 (3H, d,  
J=6Hz), 0.90 (3H, t, J=7Hz)

From the analysis of the above physical and chemical properties, and the result of the further  
investigation of identification of chemical structure, the chemical structure of the FR901379 substance has  
been identified and assigned as follows.



### Example 1

N-acyl group of FR901379 substance was eliminated by the reaction with an enzyme. In the following, this elimination process is explained in detail.

#### (1) Fermentation of *Actinoplanes utahensis*

The enzyme which is useful for eliminating N-acyl group of FR901379 Substance is produced by certain microorganisms of the Actinoplanaceae, preferably the microorganism *Actinoplanes utahensis* IFO-13244.

A stock culture of *Actinoplanes utahensis* IFO-13244 is prepared and maintained on agar slant. A loopful of the slant culture was inoculated into a seed medium consisted of starch 1%, sucrose 1%, glucose 1%, cotton seed flour 1%, peptone 0.5%, soy bean meal 0.5% and  $\text{CaCO}_3$  0.1%. The inoculated vegetative medium was incubated in a 225-ml wide mouth Erlenmeyer flask at 30 °C for about 72 hours on a rotary shaker.

This incubated vegetative medium was used directly to inoculate into a production medium consisted of sucrose 2%, peanut powder 1%,  $\text{K}_2\text{HPO}_4$  0.12%  $\text{KH}_2\text{PO}_4$  0.05% and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.025%. The inoculated production medium was allowed to ferment in a 30-liter jar fermentor at a temperature of 30 °C for about 80 hours. The fermentation medium was stirred with conventional agitators at 250 rpm and aerated at 20 liters per minute. The vegetative mycelium was collected from the fermented broth by filtration and once washed with water. The washed mycelium was directly used to eliminate N-acyl group of FR901379 substance as an enzyme source.

#### (2) Elimination Condition

FR901379 substance was dissolved in 0.25 M phosphate buffer (pH 6.5) at a concentration of 0.9 mg/ml. To a 36-liter of the solution was added a 2 kg wet weight of washed mycelium of *Actinoplanes utahensis* IFO-13244. The elimination reaction was carried out at 37 °C under for 23 hours. Reduction of FR901379 substance and increase of the deacylated FR901379 substance (hereinafter referred to as FR133303 substance) were measured using a HPLC equipped with a reverse phase column. From a 30 g of FR901379 substance, a 22.2 g of FR133303 substance was formed in the reaction mixture.

#### (3) Isolation of FR133303 Substance

The reaction mixture described above was filtered with a filter aid. The mycelial cake was discarded. The filtrate thus obtained was passed through a column of activated carbon (2 L). The column was washed with 6 L of water and eluted with 12 L of 50% aqueous acetone. The eluate was evaporated in vacuo to remove acetone and then passed through a column (4 L) of YMC GEL ODS-AM 120-S50 (Yamamura



Chemical Labs). The column was washed with water and eluted with 2% aqueous acetonitrile containing 50 mM  $\text{NaH}_2\text{PO}_4$ . Elution was monitored by analytical HPLC, using a column of LiChrospher 100 RP-18 (Cica-MERCK) and a solvent system of 3% aqueous acetonitrile containing 0.5%  $\text{NH}_4\text{H}_2\text{PO}_4$  at a flow rate of 1 ml/min, detecting the FR133303 substance with a UV monitor at 210 nm. The fractions containing the FR133303 substance were combined and passed through a column of activated carbon (400 ml). The column was washed with water and eluted with 50% aqueous acetone. The eluate was concentrated in vacuo to remove acetone and lyophilized to give 16.4 g of FR133303 substance as a white powder.

FR133303 substance has following physico-chemical properties :

Appearance :

white powder

Melting point :

150-160 °C (dec.)

Specific rotation :

$[\alpha]_D^{24} -31.17^\circ$  (C: 1.0,  $\text{H}_2\text{O}$ )

Molecular formula :

$\text{C}_{35}\text{H}_{51}\text{N}_8\text{SO}_{20}\text{Na}$

Elemental Analysis:				
Calcd : for $\text{C}_{35}\text{H}_{51}\text{N}_8\text{SO}_{20}\text{Na}$	C 43.84,	H 5.36,	N 11.69,	S 3.34 (%)
Found :	C 41.14,	H 5.74,	N 10.88,	S 3.10 (%)

Solubility :

soluble : water

slightly soluble : methanol

insoluble : n-hexane

Color reaction :

positive : iodine vapor reaction, cerium sulfate reaction, Ninhydrin reaction

negative : Molish reaction

Thin layer chromatography (TLC) :

Stationary phase	Developing solvent	Rf value
silica gel*	n-butanol:acetic acid water (3:1:2)	0.15

\* Silica Gel 60 (made by E. Merck)

Ultraviolet absorption spectrum :

$\text{H}_2\text{O}$   
 $\lambda_{\text{max}}$

$(E_{1\text{ cm}}^{1\%})$  : 201(340), 273(18), 224(sh),  
281(sh) nm

$\text{H}_2\text{O}+0.01\text{N-NaOH}$   
 $\lambda_{\text{max}}$

$(E_{1\text{ cm}}^{1\%})$  : 207(414), 243(122),  
292 (34)

Infrared absorption spectrum:

5  $\nu_{\text{max}}^{\text{KBr}}$  : 3350, 2920, 1660, 1625, 1515, 1440, 1270,  
1080, 1045, 800, 755, 715  $\text{cm}^{-1}$

$^1\text{H}$  Nuclear magnetic resonance spectrum :

10 ( $\text{D}_2\text{O}$ , 400MHz)

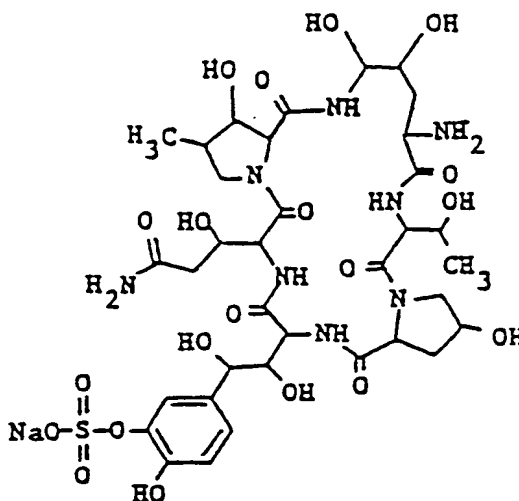
$\delta$  : 7.31 (1H, d,  $J=2\text{Hz}$ ), 7.12 (1H, dd,  $J=2\text{Hz}$  and  $8\text{Hz}$ ), 7.06 (1H, d,  $J=8\text{Hz}$ ), 5.40 (1H, d,  $J=3\text{Hz}$ ),  
5.04 (1H, d,  $J=3.5\text{Hz}$ ), 4.94 (1H, d,  $J=6\text{Hz}$ ), 4.73-4.55 (3H, m), 4.51-4.38 (4H, m), 4.31-4.23 (3H,  
m), 4.11-4.06 (2H, m), 3.94-3.89 (2H, m), 3.41 (1H, m), 2.60-2.34 (5H, m), 2.14 (1H, m), 2.03 (1H,  
m), 1.28 (3H, d,  $J=6\text{Hz}$ ), 1.01 (3H, d,  $J=6.5\text{Hz}$ )

15  $^{13}\text{C}$  Nuclear magnetic resonance spectrum :

( $\text{D}_2\text{O}$ , 100MHz)

$\delta$  : 178.3 (s), 175.9 (s), 174.3 (s), 174.2 (s), 174.0 (s), 171.8 (s), 171.3 (s), 150.9 (s), 141.5 (s), 134.4  
(s), 128.2 (d), 124.5 (d), 120.3 (d), 78.1 (d), 77.0 (d), 76.9 (d), 76.6 (d), 72.9 (d), 72.8 (d), 71.2 (d),  
69.3 (d), 69.2 (d), 63.7 (d), 60.1 (d), 58.3 (t), 58.0 (d), 56.9 (d), 55.3 (d), 54.7 (t), 41.8 (t), 39.7 (d),  
20 39.5 (t), 33.5 (t), 21.4 (q), 13.3 (q)

The chemical structure of FR133303 substance has been identified and assigned as follows.



## Example 2

45 (1) A solution of 4-hydroxybenzoic acid (19.2 g) in 10% NaOH (120 ml) was dropwise added to 480 ml of dimethyl sulfoxide over 30 minutes during which the temperature in reaction mixture was controlled between 30 and  $40^\circ\text{C}$ . After adding, the solution was cooled to  $17-20^\circ\text{C}$ . 1-Bromooctane (28.95 g) was dropwise added to the solution over 30 minutes and the reaction mixture was vigorously stirred for 4 hours at room temperature. The reaction mixture was poured into ice water (1200 ml) and acidified with 40 ml of conc.  
50 hydrochloric acid. After vigorously stirring for another 1 hour, the resulting solid was removed by filtration and dissolved in 60 ml of acetonitrile. The solution was refluxed over 30 minutes and was allowed to stand overnight at room temperature to yield 4-octyloxybenzoic acid (13.8 g) as a crystal (MP  $96^\circ\text{C}$ , Anal Calcd. for  $\text{C}_{15}\text{H}_{22}\text{O}_3$  : C 71.97, H 8.86, Found : C 71.30, H 8.89).

To a solution of 4-octyloxybenzoic acid (13.8 g) in diethyl ether (552 ml) were added 2,4,5-trichlorophenol (10.87 g) and N,N'-dicyclohexylcarbodiimide (11.37 g). The solution was stirred under a nitrogen atmosphere for 18 hours at room temperature. The precipitate was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in petroleum ether and was allowed to stand on ice-water. The resulting crystals (15.2 g) were filtered and dissolved in warm n-hexane (150 ml). After standing

overnight at room temperature, the resulting crystal was removed by filtration. The filtrate was concentrated to an oil which was purified by a column chromatography over silica gel using a mixture of ethyl acetate and n-hexane to give 2,4,5-trichlorophenyl 4-octyloxybenzoate (7.58 g)(MP 53°C, Anal Calcd. for  $C_{21}H_{23}O_3Cl_3$  : Cl 24.75, Found : Cl 24.05).

- 5 (2) To a solution of FR133303 substance (2.04 g) in N,N-dimethylformamide (60 ml) were added 2,4,5-trichlorophenyl 4-octyloxybenzoate (2.04 g) and 4-dimethylaminopyridine (0.283 g). The solution was stirred under a nitrogen atmosphere at room temperature for 15 hours. 4-Dimethylaminopyridine (0.20 g) was added to the solution and mixture was stirred for another 24 hours. The reaction mixture was poured into water (600 ml) and the pH was adjusted to 6.0. The mixture was washed twice with an equal volume of ethyl acetate and concentrated to 30 ml. The concentrate was applied on a column (150 ml) of DEAE-Toyopearl (Cl type, manufactured by Tosoh). The column was washed with 50% aqueous methanol and developed with 50% aqueous methanol containing 1M sodium chloride aqueous solution. The elution of product was assessed by the same HPLC system as described in Example 1(3) except that the concentration of acetonitrile in solvent was 40%. The fractions containing the object compound were pooled and evaporated in vacuo to remove methanol. The solution was absorbed on a column (1 L) of YMC GEL ODS-AM 120-S50 in order to remove salt. The column was washed with water and eluted with 30% aqueous acetonitrile. The eluate was evaporated in vacuo to remove acetonitrile and lyophilized to give the object compound (hereinafter referred to as FR131535 substance) (1.4 g) as a white powder.

FR131535 substance has following physico-chemical properties :

- 20 Appearance :

white powder

Melting point :

170-189°C (dec.)

- 25 Specific rotation :

$[\alpha]_D^{20}$  -14.4° (C: 10, H<sub>2</sub>O)

Molecular formula :

$C_{50}H_{71}N_8SO_{22}Na$

30

Elemental Analysis :					
Calcd :for $C_{50}H_{71}N_8SO_{22}Na \cdot 6H_2O$	C 46.22,	H 6.44,	N 8.62,	S 2.46,	Na 1.77 (%)
Found :	C 46.80,	H 6.13,	N 8.78,	S 1.96,	Na 1.81 (%)

35

Solubility :

soluble : methanol, water

slightly soluble : acetone

insoluble : n-hexane

Color reaction :

40

positive : iodine vapor reaction, cerium sulfate reaction

45

50

55

Thin layer chromatography (TLC) :

Stationary phase	Developing solvent	R <sub>f</sub> value
silica gel*	n-butanol:acetic acid: water (6:1:1)	0.21

\* Silica Gel 60 (made by E. Merck)

Infrared absorption spectrum :

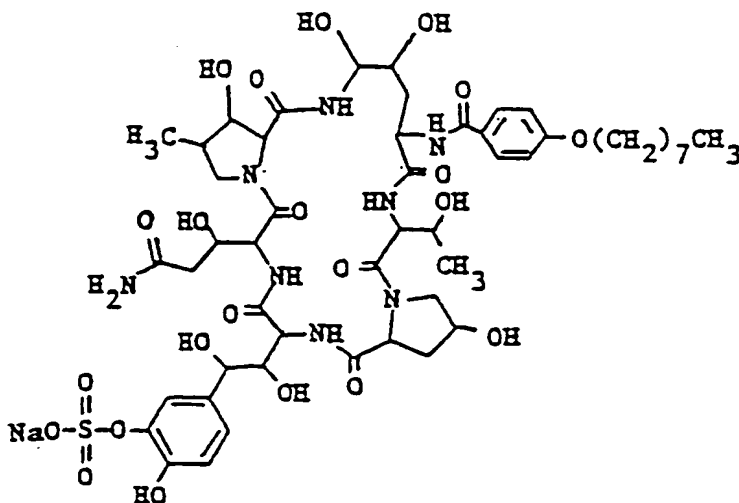
$\nu_{\text{max}}^{\text{KBr}}$  : 3330, 2900, 2850, 1620, 1500, 1430, 1270,  
1250, 1170, 1110, 1080, 1040, 960, 940,  
880, 840, 800, 750, 710  $\text{cm}^{-1}$

$^1\text{H}$  Nuclear magnetic resonance spectrum :

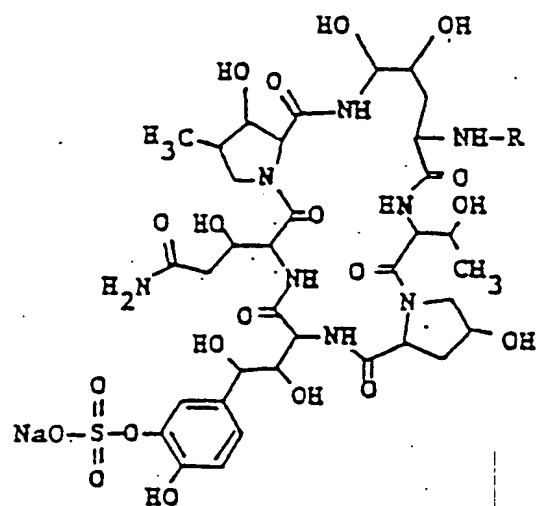
( $\text{CD}_3\text{OD}$ , 200MHz)

$\delta$  : 7.78 (2H, d,  $J=8\text{Hz}$ ), 7.31 (1H, d,  $J=2\text{Hz}$ ), 7.03 (1H, dd,  $J=2\text{Hz}$  and  $8\text{Hz}$ ), 6.96 (2H, d,  $J=8\text{Hz}$ ),  
6.87 (1H, d,  $J=8\text{Hz}$ ), 5.33 (1H, d,  $J=3\text{Hz}$ ), 5.08 (1H, d,  $J=4\text{Hz}$ ), 4.99 (1H, d,  $J=3\text{Hz}$ ), 4.80-3.20  
(17H, m), 2.83 (1H, m), 2.65-2.30 (4H, m), 2.22-1.90 (2H, m), 1.79 (2H, m), 1.56-1.25 (10H, m),  
1.19 (3H, d,  $J=6\text{Hz}$ ), 1.06 (3H, d,  $J=6.5\text{Hz}$ ), 0.90 (3H, t,  $J=6.5\text{Hz}$ )

The chemical structure of FR131535 substance has been identified and assigned as follows.



In the following, the structures of the compounds of Examples 3 to 11 are shown.



Example No.	Compound No.	R
3	FR138260	$\begin{array}{c} \text{(D)} \\ \text{---COCH---} \langle \text{benzene ring} \rangle \text{---O(CH}_2\text{)}_7\text{CH}_3 \\   \\ \text{NHCOO}^t\text{Bu} \end{array}$
4	FR138727	$\begin{array}{c} \text{(D)} \\ \text{---COCH---} \langle \text{benzene ring} \rangle \text{---O(CH}_2\text{)}_7\text{CH}_3 \\   \\ \text{NH}_2 \end{array}$
5	FR138364	$\begin{array}{c} \text{(L)} \\ \text{---COCHCH}_2\text{---} \langle \text{benzene ring} \rangle \text{---O(CH}_2\text{)}_7\text{CH}_3 \\   \\ \text{NHCOO}^t\text{Bu} \end{array}$
6	FR138261	$\text{---COO}^t\text{Bu}$
7	FR138363	$\text{---COCH}_3$

8	FR138728	$-\text{COCH}_2\text{Br}$
9	FR138538	$-\text{COO}-\text{C}_6\text{H}_5$
10	FR138539	$  \begin{array}{c}  \text{---COC---} \\  \parallel \\  \text{CH}_3\text{O-N}  \end{array}  \begin{array}{c}  \text{---} \\  \diagup \quad \diagdown \\  \text{S} \quad \text{N} \\  \quad \quad \quad \diagup \quad \diagdown \\  \quad \quad \quad \text{CH}_2 \quad \text{NH}_2  \end{array}  $
11	FR138365	$-\text{O}_2\text{S}-\text{C}_6\text{H}_4-\text{CH}_3$

### Example 3

To a solution of FR133303 substance (1 g) and N-(t-butoxycarbonyl)-D-2-(p-octyloxyphenyl)glycine succinimido ester (0.596 g) in N,N-dimethylformamide (3 ml) was added 4-dimethylaminopyridine (0.165g). The mixture was stirred for 12 hours at room temperature. The reaction mixture was added to water (30 ml) and then adjusted to pH 6. The aqueous solution was washed with ethyl acetate, and subjected to ion exchange chromatography on DEAE-Toyopearl ( $\text{Cl}^-$ ) (60 ml) and eluted with 50% methanol in 1M aqueous solution of sodium chloride. The fractions containing the object compound were combined and evaporated under reduced pressure to remove methanol. The aqueous solution was adjusted to pH 4.5 with 1N hydrochloric acid and subjected to column chromatography on Diaion HP-20 (Trademark, Manufactured by Mitsubishi Chemical Industries) (130 ml) and eluted with 80% aqueous methanol. The fractions containing the object compound were combined and evaporated under reduced pressure to remove methanol. The residue was lyophilized to give object acylated compound (hereinafter referred to as FR138260 substance) (0.77 g).

IR (Nujol) : 3300, 1660, 1500, 1240, 1045, 800, 720  $\text{cm}^{-1}$

NMR ( $\text{CD}_3\text{OD}$ ,  $\delta$ ) : 0.92 (3H, t,  $J=6.8\text{Hz}$ ), 1.05 (3H, d,  $J=6.8\text{Hz}$ ), 1.17-1.33 (13H, m), 1.43 (9H, s), 1.6-1.8 (2H, m), 1.9-2.1 (3H, m), 2.50 (3H, m), 2.75 (1H, dd,  $J=16\text{Hz}$  and  $4\text{Hz}$ ), 3.35 (1H, m), 3.7-3.8 (1H, m), 3.93 (2H, t,  $J=6.2\text{Hz}$ ), 3.9-4.2 (5H, m), 4.3-4.5 (5H, m), 4.5-4.7 (3H, m), 4.97 (1H, d,  $J=3\text{Hz}$ ), 5.05 (1H, d,  $J=4\text{Hz}$ ), 5.11 (1H, s), 5.30 (1H, d,  $J=3\text{Hz}$ ), 6.85 (1H, d,  $J=8.3\text{Hz}$ ), 6.86 (2H, d,  $J=8.6\text{Hz}$ ), 7.02 (1H, d,  $J=8.3\text{Hz}$ ), 7.26 (2H, d,  $J=8.6\text{Hz}$ ), 7.31 (1H, s)

FAB-MS :  $m/z = 1343$  (M + Na)

### Example 4

FR138260 substance obtained in Example 3 (0.25 g) was added to trifluoroacetic acid (1.25 ml) and stirred for 10 minutes. The reaction mixture was added to water (30 ml) and then adjusted to pH 4.5 with saturated aqueous solution of sodium bicarbonate. The aqueous solution was subjected to column chromatography on Diaion HP-20 (100 ml) and eluted with 80% aqueous methanol. The fractions containing the object compound were combined and evaporated under reduced pressure to remove methanol. The residue was lyophilized to give the object compound (hereinafter referred to as FR138727 substance) (15 mg).

NMR ( $\text{CD}_3\text{OD}$ ,  $\delta$ ) : 0.90 (3H, t,  $J=6.8\text{Hz}$ ), 1.05 (3H, d,  $J=6.8\text{Hz}$ ), 1.17-1.33 (13H, m), 1.6-1.8 (2H, m), 1.9-2.1 (3H, m), 2.50 (1H, m), 2.75 (1H, dd,  $J=16\text{Hz}$  and  $4\text{Hz}$ ), 3.40 (1H, m), 3.7-3.8 (1H, m), 3.98 (2H, t,  $J=6.2\text{Hz}$ ), 3.9-4.2 (5H, m), 4.3-4.5 (5H, m), 4.5-4.7 (3H, m), 4.97 (1H, d,  $J=3\text{Hz}$ ), 5.06 (1H, s), 5.20 (1H, d,  $J=3\text{Hz}$ ), 5.40 (1H, d,  $J=3\text{Hz}$ ), 6.85 (1H, d,  $J=8.3\text{Hz}$ ), 6.95 (2H, d,  $J=8.5\text{Hz}$ ), 7.02 (1H, d,  $J=8.3\text{Hz}$ ), 7.30 (1H, d,  $J=8.5\text{Hz}$ ), 7.44 (1H, s)

FAB-MS :  $e/z = 1259 (M + K)$

### Example 5

- 5 FR138364 substance was obtained by reacting FR133303 substance with O<sup>4</sup>-octyl-N-(t-butoxycarbonyl)-L-tyrosine succinimido ester according to a similar manner to that of

### Example 3.

- 10 IR (Nujol) : 3300, 1660, 1620, 1240, 1050 cm<sup>-1</sup>  
 NMR (CD<sub>3</sub>OD,  $\delta$ ) : 0.904 (3H, t, J=6.8Hz), 1.06 (3H, d, J=6.8Hz), 1.17 (3H, d, J=6.7Hz), 1.20-1.30 (10H, m), 1.35 (9H, s), 1.74 (2H, quintet, J=6.5Hz), 1.9-2.1 (3H, m), 2.45 (3H, m), 2.76 (1H, dd, J=16Hz and 4Hz), 3.0-3.1 (2H, m), 3.37 (1H, m), 3.77 (1H, d, J=11Hz), 3.92 (2H, t, J=6.8Hz), 3.9-4.2 (7H, m), 4.3-4.5 (5H, m), 4.5-4.6 (3H, m),  
 15 4.94 (1H, d, J=3Hz), 5.05 (1H, d, J=3.8Hz), 5.31 (1H, d, J=3Hz), 6.79 (2H, d, J=8.5Hz), 6.85 (1H, d, J=8.3Hz), 7.03 (1H, dd, J=8.3Hz and 2Hz), 7.12 (2H, d, J=8.5Hz), 7.31 (1H, d, J=2Hz)  
 FAB-MS :  $e/z = 1357 (M + Na)$

### 20 Example 6

- A solution of FR133303 substance (0.5 g) in a mixture of water (5 ml) and tetrahydrofuran (5 ml) was adjusted to pH 7 with saturated aqueous solution of sodium bicarbonate and N,N-di-t-butylcarbonate (0.114 g) was added thereto at room temperature. The mixture was stirred for 5 hours at room temperature  
 25 maintaining pH 7 with saturated aqueous solution of sodium bicarbonate. The reaction mixture was added to water and adjusted to pH6. The aqueous solution was washed with ethyl acetate, and subjected to ion exchange chromatography on DEAE-Toyopearl (C1<sup>-</sup>) (30 ml) and eluted with 50% methanol in 1M aqueous solution of sodium chloride. The fractions containing the object compound were combined and evaporated under reduced pressure to remove methanol. The aqueous solution was adjusted to pH 4.5 with 1N  
 30 hydrochloric acid and subjected to column chromatography on Diaion HP-20 (100 ml) and eluted with 80% aqueous methanol. The fractions containing the object compound were combined and evaporated under reduced pressure to remove methanol. The residue was lyophilized to give the object acylated compound (hereinafter referred to as FR138261 substance) (0.145 g).

- IR (Nujol) : 3300, 1660, 1620, 1240, 1050 cm<sup>-1</sup>  
 35 NMR (CD<sub>3</sub>OD,  $\delta$ ) : 1.06 (3H, d, J=6.8Hz), 1.18 (3H, d, J=6.0Hz), 1.40 (9H, s), 1.9-2.1 (3H, m), 2.44 (3H, m), 2.82 (1H, dd, J=16Hz and 4Hz), 3.37 (1H, m), 3.75 (1H, d, J=11Hz), 3.89-4 (2H, m), 4.10 (1H, m), 4.15 (1H, m), 4.29 (1H, dd, J=6Hz and 2Hz), 4.36-4.45 (5H, m), 4.5-4.6 (3H, m), 4.97 (1H, d, J=3Hz), 5.06 (1H, dd, J=8.2Hz and 4Hz), 5.33 (1H, d, J=3Hz), 6.85 (1H, d, J=8.3Hz), 7.03 (1H, dd, J=8.3Hz and 2Hz), 7.30 (1H, d, J=2Hz), 7.50 (1H, d, J=8.2Hz)  
 40 FAB-MS :  $e/z = 1081 (M + Na)$

### Example 7

- 45 FR138363 substance was obtained by reacting FR133303 substance with acetyl chloride according to a similar manner to that of Example 6.

- IR (Nujol) : 3300, 1620, 1250, 1040 cm<sup>-1</sup>  
 NMR (CD<sub>3</sub>OD,  $\delta$ ) : 1.06 (3H, d, J=6.8Hz), 1.20 (3H, d, J=6Hz), 1.78-2.05 (3H, m), 1.96 (3H, s), 2.21-2.54 (3H, m), 2.95 (1H, m), 3.35-3.42 (1H, m), 3.58-4.42 (11H, m), 4.50-5.05 (5H, m), 5.23 (1H, m), 6.88 (1H, d, J=8.3Hz), 7.05 (1H, dd, J=8.3Hz and 2Hz), 7.35 (1H, d, J=2Hz)  
 50 FAB-MS : 1023 (M + Na)

### Example 8

- 55 FR138728 substance was obtained by reacting FR133303 substance with 2-bromoacetyl chloride according to a similar manner to that of Example 6.

IR (Nujol) : 3300, 1660, 1620, 1500, 1220, 1040 cm<sup>-1</sup>

NMR (CD<sub>3</sub>OD,  $\delta$ ) : 1.06 (3H, d, J = 6.9Hz), 1.17 (3H, d, J = 6.1Hz), 1.9-2.1 (3H, m), 2.50 (3H, m), 2.80 (1H, dd, J = 16Hz and 4Hz), 3.37 (1H, m), 3.6-4.0 (5H, m), 4.09 (1H, m), 4.16 (1H, m), 4.29 (1H, dd, J = 6Hz and 2Hz), 4.36-4.45 (5H, m), 4.5-4.7 (3H, m), 4.97 (1H, d, J = 3Hz), 5.04 (1H, dd, J = 8.6Hz and 4Hz), 5.25 (1H, d, J = 3.1Hz), 6.85 (1H, d, J = 8.3Hz), 7.03 (1H, dd, J = 8.3Hz and 2.1Hz), 7.31 (1H, d, J = 2Hz), 7.52 (1H, d, J = 8.6Hz)

FAB-MS : e/z = 1103 (M + Na)

#### Example 9

FR138538 substance was obtained by reacting FR133303 substance with benzoyl chloride according to a similar manner to that of Example 6.

IR (Nujol) : 3300, 1640, 1240 cm<sup>-1</sup>

NMR (CD<sub>3</sub>OD,  $\delta$ ) : 1.05 (3H, d, J = 6.8Hz), 1.18 (3H, d, J = 6Hz), 1.89-2.12 (3H, m), 2.31-2.53 (3H, m), 2.75 (1H, dd, J = 12Hz and 4Hz), 3.38 (1H, m), 3.76 (1H, d, J = 11Hz), 3.87-3.98 (1H, m), 4.02-4.18 (2H, m), 4.22-4.32 (4H, m), 4.37-4.40 (3H, m), 4.49-4.62 (3H, m), 4.98 (1H, m), 5.02 (1H, m), 5.37 (1H, d, J = 3Hz), 6.85 (1H, d, J = 8.3Hz), 7.04 (1H, dd, J = 8.3Hz and 2Hz), 7.11-7.50 (6H, m)

FAB-MS : e/z = 1101 (M + Na)

#### Example 10

FR138539 substance was obtained by reacting FR133303 substance with 2-(2-aminothiazol-4-yl)-2-methoxyiminoacetic acid according to a similar manner to that of Example 6.

IR (Nujol) : 3300, 1650, 1620, 1520, 1260, 1040 cm<sup>-1</sup>

NMR (CD<sub>3</sub>OD,  $\delta$ ) : 1.05 (3H, d, J = 6.8Hz), 1.21 (3H, d, J = 5.9Hz), 1.89-2.21 (3H, m), 2.29-2.61 (3H, m), 2.78-2.89 (1H, m), 3.32-3.42 (1H, m), 3.76-3.82 (1H, m), 3.91-4.01 (2H, m), 3.95 (3H, s), 4.13 (1H, m), 4.16 (1H, m), 4.24-4.27 (1H, m), 4.32-4.43 (5H, m), 4.46-4.62 (3H, m), 4.97-4.99 (1H, m), 5.08 (1H, m), 5.41 (1H, m), 6.79 (1H, s), 6.86 (1H, d, J = 8.1Hz), 7.04 (1H, dd, J = 8.1Hz and 2Hz), 7.31 (1H, d, J = 2Hz), 7.51 (1H, d, J = 7Hz)

FAB-MS : e/z = 1143 (M<sup>+</sup>)

#### Example 11

FR138365 substance was obtained by reacting FR133303 substance with tosyl chloride according to a similar manner to that of Example 6.

IR (Nujol) : 3300, 1650, 1620, 1260, 1060 cm<sup>-1</sup>

NMR (CD<sub>3</sub>OD,  $\delta$ ) : 0.75 (3H, d, J = 6.8Hz), 1.07 (3H, d, J = 6.0Hz), 1.61-1.79 (1H, m), 1.91-2.05 (3H, m), 2.30-2.59 (3H, m), 3.36 (1H, m), 3.68 (1H, d, J = 11Hz), 3.81-4.07 (4H, m), 4.22 (1H, m), 4.32-4.40 (5H, m), 4.42-4.60 (3H, m), 4.7 (1H, m), 5.0 (1H, m), 5.42 (1H, d, J = 3Hz), 6.85 (1H, d, J = 8.3Hz), 7.03 (1H, dd, J = 8.3Hz and 2Hz), 7.29-7.33 (3H, m), 7.75 (1H, d, J = 8.3Hz)

FAB-MS : e/z = 1135 (M + Na)

#### Preparation 11

To a solution of 6-hydroxy-2-naphthoic acid (1 g) in the mixture of 10 % sodium hydroxide aqueous solution (4.25 ml) and dimethylsulfoxide (17 ml) was added octyl bromide (0.918 ml). The mixture was stirred for 6 hours at 60 °C.

The reaction mixture was added to a mixture of water and ethyl acetate and adjusted to pH 3 with conc. hydrochloric acid. The organic layer was separated and dried over magnesium sulfate. The magnesium sulfate was filtered off, and the filtrate was evaporated under reduced pressure to give 6-octyloxy-2-naphthoic acid (0.91 g).

IR (Nujol) : 1670, 1620, 1210 cm<sup>-1</sup>

NMR (DMSO-d<sub>6</sub>,  $\delta$ ) : 0.86 (3H, t, J = 6.7 Hz), 1.2 - 1.6 (10H, m), 1.78 (2H, m), 4.10 (2H, t, J = 6.7 Hz), 7.19 (1H, dd, J = 2.3 and 8.8 Hz), 7.36 (1H, d, J = 2.3 Hz), 7.83 (1H, d, J = 8.8 Hz), 7.97 (2H, d, J = 8.8 Hz), 8.52 (1H, s)



### Preparation 12

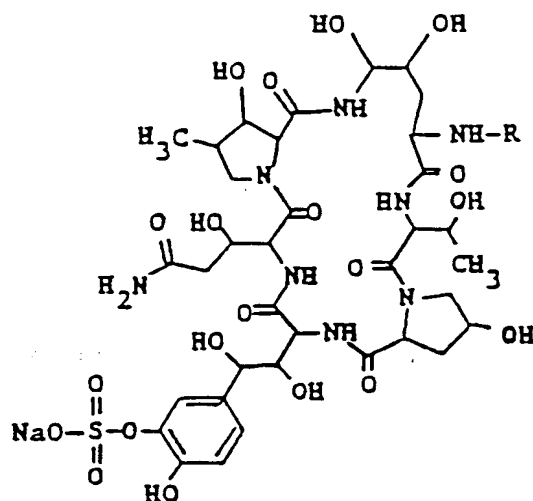
1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.703 g) was added to a solution of 6-octyloxy-2-naphthoic acid (0.85 g) and 1-hydroxy-1H-benzotriazole (0.382 g) in ethyl acetate (26 ml). The mixture was stirred for two hours at room temperature.

The reaction mixture was added to water and the separated organic layer was washed with water and sodium chloride aqueous solution. Then the organic layer was dried over magnesium sulfate. The magnesium sulfate was filtered off, and the filtrate was evaporated under reduced pressure to give 1-(6-octyloxy-2-naphthoyl)-1H-benzotriazole-3-oxide (0.74 g).

IR (Nujol) : 1770, 1740, 1620, 1190, 1020, 740  $\text{cm}^{-1}$

NMR ( $\text{CDCl}_3$ ,  $\delta$ ) : 0.90 (3H, t,  $J=6.8$  Hz), 1.2 - 1.6 (10H, m), 1.89 (2H, m), 4.14 (2H, t,  $J=6.8$  Hz), 7.1 - 7.3 (2H, m), 7.4 - 7.6 (3H, m), 7.8 - 8.0 (2H, m), 8.1 - 8.2 (2H, m), 8.80 (1H, s)

In the following, the structure of the compound of Example 12 is shown.



Example No.	Compound No.	R
12	FR139687	

### Example 12

To a solution of FR133303 substance (0.5 g) and 1-(6-octyloxy-2-naphthoyl)-1H-benzotriazole-3-oxide (0.271 g) in N,N-dimethylformamide (1.5 ml) was added 4-dimethylaminopyridine (0.0828 g). The mixture was stirred for 12 hours at room temperature.

The reaction mixture was added to water and adjusted to pH 6. The aqueous solution was washed with ethyl acetate, and subjected to ion exchange chromatography on DEAE-Toyopearl ( $\text{Cl}^-$ ) (30 ml) and eluted with 50 % methanol in 1M sodium chloride solution. The fractions containing the object compound were combined and evaporated under reduced pressure to remove methanol. The aqueous solution was adjusted to pH 4.5 with 1N hydrochloric acid and subjected to column chromatography on Diaion HP-20 (65 ml) and eluted with 80 % aqueous methanol. The fractions containing the object compound were combined and evaporated under reduced pressure to remove methanol. The residue was lyophilized to give object acylated compound (hereinafter referred to as FR139687 substance) (0.214 g).

IR (Nujol) : 3300, 1620, 1500  $\text{cm}^{-1}$

NMR (DMSO- $d_6$  + D<sub>2</sub>O,  $\delta$ ) : 0.86 (3H, t, J=6.8 Hz), 0.97 (3H, d, J=6.8 Hz), 1.06 (3H, d, J=6.8 Hz), 1.2 - 1.5 (10H, m), 1.6 - 2.0 (5H, m), 2.2 - 2.5 (3H, m), 2.4 - 2.6 (1H, m), 3.18 (1H, m), 3.6 - 3.9 (1H, m), 4.0 - 4.6 (15H, m), 4.84 (1H, d, J=3 Hz), 4.90 (1H, d, J=3 Hz), 5.11 (1H, d, J=3 Hz), 6.76 (1H, d, J=8.3 Hz), 6.93 (1H, d, J=8.3 Hz), 7.13 (1H, s), 7.25 (1H, d, J=8.3 Hz), 7.39 (1H, s), 7.8 - 8.0 (3H, m), 8.44 (1H, s)

FAB-MS  $m/z$  = 1264 (M + Na)

The following compounds (Preparations 13 to 16) were obtained according to a similar manner to that of Preparation 5.

#### Preparation 13

N-(t-Butoxycarbonyl)-L-2-(2-naphthyl)glycine succinimido ester

IR (Nujol) : 3350, 1800, 1770, 1730, 1680, 1500, 1200  $\text{cm}^{-1}$

#### Preparation 14

Succinimido 2-(4-biphenyl)acetate

IR (Nujol) : 1800, 1770, 1720, 1200  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.82 (4H, s), 4.17 (2H, s), 7.30-7.50 (5H, m), 7.45 (2H, d, J=8.1Hz), 7.67 (2H, d, J=8.1Hz)

#### Preparation 15

Succinimido 4-t-butylbenzoate

IR (Nujol) : 1760, 1730, 1200, 1070, 990  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.33 (9H, s), 2.89 (4H, s), 7.68 (2H, d, J=8.5Hz), 8.03 (2H, d, J=8.5Hz)

#### Preparation 16

Succinimido 4-(4-phenylbutoxy)benzoate

IR (Nujol) : 1730, 1600, 1240, 1170, 1070  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.75 (4H, m), 2.65 (2H, m), 4.14 (2H, m), 7.15 (2H, d, J=8.9Hz), 7.13-7.35 (5H, m), 8.03 (2H, d, J=8.9Hz)

#### Preparation 17

To neat 3,7-dimethyloctanol (5 ml) was added phosphorus tribromide (1.01 ml). The mixture was stirred for 4 hours at 60°C. The reaction mixture was added to a mixture of water and n-hexane. The organic layer was separated and dried over magnesium sulfate. The magnesium sulfate was filtered off, and the filtrate was evaporated under reduced pressure to give 3,7-dimethyloctyl bromide (4.40 g).

IR (Neat) : 2900, 1450  $\text{cm}^{-1}$

NMR (CDCl<sub>3</sub>,  $\delta$ ) : 0.87 (6H, d, J=6.6Hz), 0.89 (3H, d, J=6.4Hz), 1.1-1.3 (6H, m), 1.5-1.9 (4H, m), 3.3-3.5 (2H, m)

The following compounds (Preparations 18 to 23) were obtained according to a similar manner to that of Preparation 11.

#### Preparation 18

4-[4-(Octyloxy)phenoxy]benzoic acid

IR (Nujol) : 1680, 1600, 1240, 840  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.87 (3H, t, J=6.7Hz), 1.1-1.6 (10H, m), 1.71 (2H, m), 3.96 (2H, t, J=6.4Hz), 6.9-7.1 (6H, m), 7.92 (2H, d, J=8.7Hz), 12.8 (1H, br s)

#### Preparation 19

6-(Butoxy)-2-naphthoic acid

IR (Nujol) : 1660, 1610, 1205  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.96 (3H, t,  $J=7.29\text{Hz}$ ), 1.48 (2H, qt,  $J=7.29\text{Hz}$  and  $7\text{Hz}$ ), 1.78 (2H, tt,  $J=7\text{Hz}$  and  $6.45\text{Hz}$ ), 4.12 (2H, t,  $J=6.45\text{Hz}$ ), 7.24 (1H, dd,  $J=9.0\text{Hz}$  and  $2.3\text{Hz}$ ), 7.40 (1H, d,  $J=2.3\text{Hz}$ ), 7.86 (1H, d,  $J=8.7\text{Hz}$ ), 7.94 (1H, d,  $J=8.7\text{Hz}$ ), 8.01 (1H, d,  $J=9.0\text{Hz}$ ), 8.52 (1H, s)

#### Preparation 20

6-Decyloxy-2-naphthoic acid

IR (Nujol) : 1670, 1620, 1210  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.85 (3H, t,  $J=6.7\text{Hz}$ ), 1.2-1.6 (14H, m), 1.78 (2H, m), 4.11 (2H, t,  $J=6.4\text{Hz}$ ), 7.23 (1H, dd,  $J=8.9\text{Hz}$  and  $2.4\text{Hz}$ ), 7.39 (1H, d,  $J=2.4\text{Hz}$ ), 7.86 (1H, d,  $J=8.7\text{Hz}$ ), 7.93 (1H, d,  $J=8.7\text{Hz}$ ), 8.01 (1H, d,  $J=8.9\text{Hz}$ ), 8.5 (1H, s)

#### Preparation 21

6-Hexyloxy-2-naphthoic acid

IR (Nujol) : 1660, 1620, 1290, 1210  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.89 (3H, t,  $J=6.8\text{Hz}$ ), 1.2-1.6 (6H, m), 1.78 (2H, quint,  $J=6.5\text{Hz}$ ), 4.11 (2H, t,  $J=6.5\text{Hz}$ ), 7.23 (1H, dd,  $J=9.0\text{Hz}$  and  $2.4\text{Hz}$ ), 7.39 (1H, d,  $J=2.4\text{Hz}$ ), 7.86 (1H, d,  $J=8.7\text{Hz}$ ), 7.94 (1H, d,  $J=8.7\text{Hz}$ ), 8.01 (1H, d,  $J=9.0\text{Hz}$ ), 8.52 (1H, s)

#### Preparation 22

6-Dodecyloxy-2-naphthoic acid

IR (Nujol) : 1670, 1620, 1210  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.85 (3H, t,  $J=6.7\text{Hz}$ ), 1.20-1.60 (18H, m), 1.78 (2H, m), 4.11 (2H, t,  $J=6.5\text{Hz}$ ), 7.22 (1H, dd,  $J=9.0\text{Hz}$  and  $2.4\text{Hz}$ ), 7.39 (1H, d,  $J=2.4\text{Hz}$ ), 7.85 (1H, d,  $J=8.7\text{Hz}$ ), 7.93 (1H, d,  $J=8.7\text{Hz}$ ), 8.00 (1H, d,  $J=9.0\text{Hz}$ ), 8.51 (1H, s), 12.90 (1H, s)

#### Preparation 23

6-(3,7-Dimethyloctyloxy)-2-naphthoic acid

IR (Nujol) : 1660, 1610, 1290, 1210  $\text{cm}^{-1}$

NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.84 (6H, d,  $J=6.6\text{Hz}$ ), 0.94 (3H, d,  $J=6.1\text{Hz}$ ), 1.1-1.4 (6H, m), 1.4-1.9 (4H, m), 4.15 (2H, t,  $J=6.7\text{Hz}$ ), 7.22 (1H, dd,  $J=9.0\text{Hz}$  and  $2.4\text{Hz}$ ), 7.41 (1H, d,  $J=2.4\text{Hz}$ ), 7.86 (1H, d,  $J=8.6\text{Hz}$ ), 7.93 (1H, d,  $J=8.6\text{Hz}$ ), 8.01 (1H, d,  $J=9.0\text{Hz}$ ), 8.52 (1H, s)

The following compounds (Preparations 24 to 31) were obtained according to a similar manner to that of

#### Preparation 12.

#### Preparation 24

1-[4-(4-Octyloxy)phenoxy]benzoyl-1H-benzotriazole-3-oxide

IR (Nujol) : 1770, 1730, 1600, 1500, 1230, 980  $\text{cm}^{-1}$

#### Preparation 25

1-(6-Butoxy-2-naphthoyl)-1H-benzotriazole-3-oxide

IR (Nujol) : 1760, 1610, 1260, 1180, 1020  $\text{cm}^{-1}$

#### Preparation 26

1-(6-Decyloxy-2-naphthoyl)-1H-benzotriazole-3-oxide

IR (Nujol) : 1780, 1620, 1190, 1000  $\text{cm}^{-1}$

#### Preparation 27

## 1-(6-Hexyloxy-2-naphthoyl)-1H-benzotriazole-3-oxide

IR (Nujol) : 1780, 1610, 1190  $\text{cm}^{-1}$ 

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.89 (3H, t,  $J=6.7\text{Hz}$ ), 1.2-1.6 (6H, m), 1.79 (2H, m), 4.12 (2H, t,  $J=6.5\text{Hz}$ ), 7.24 (1H, dd,  $J=9.0\text{Hz}$  and  $2.4\text{Hz}$ ), 7.39 (1H, d,  $J=2.4\text{Hz}$ ), 7.41 (1H, t,  $J=8\text{Hz}$ ), 7.54 (1H, t,  $J=8\text{Hz}$ ), 7.72 (1H, d,  $J=8\text{Hz}$ ), 7.88 (1H, d,  $J=8.7\text{Hz}$ ), 7.90 (1H, d,  $J=8.7\text{Hz}$ ), 7.97 (1H, d,  $J=8\text{Hz}$ ), 8.02 (1H, d,  $J=9.0\text{Hz}$ ), 8.51 (1H, s)

Preparation 28

## 1-(6-Dodecyloxy-2-naphthoyl)-1H-benzotriazole-3-oxide

IR (Nujol) : 1770, 1620, 1190, 1030, 730  $\text{cm}^{-1}$ 

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.85 (3H, t,  $J=6.7\text{Hz}$ ), 1.2-1.3 (18H, m), 1.78 (2H, m), 4.11 (2H, t,  $J=6.5\text{Hz}$ ), 7.22 (1H, dd,  $J=9.0\text{Hz}$  and  $2.4\text{Hz}$ ), 7.39 (1H, d,  $J=2.4\text{Hz}$ ), 7.40 (1H, t,  $J=8\text{Hz}$ ), 7.55 (1H, t,  $J=8\text{Hz}$ ), 7.73 (1H, d,  $J=8\text{Hz}$ ), 7.85 (1H, d,  $J=8.7\text{Hz}$ ), 7.93 (1H, d,  $J=8.7\text{Hz}$ ), 7.99 (1H, d,  $J=8\text{Hz}$ ), 8.00 (1H, d,  $J=9.0\text{Hz}$ ), 8.51 (1H, s)

Preparation 29

## 1-[6-(3,7-Dimethyloctyloxy)-2-naphthoyl]-1H-benzotriazole-3-oxide

IR (Nujol) : 1780, 1620, 1190  $\text{cm}^{-1}$ Preparation 30

## 1-[(2E,6E)-3,7,11-Trimethyl-2,6,10-dodecatrienoyl]-1H-benzotriazole-3-oxide

IR (Neat) : 2900, 1780, 1620, 1420, 1070  $\text{cm}^{-1}$ Preparation 31

3,7-Dimethyl-6-octenyl bromide was obtained according to a similar manner to that of Preparation 17.

IR (Neat) : 2900, 1440, 1380  $\text{cm}^{-1}$ 

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, d,  $J=6.3\text{Hz}$ ), 1.0-1.5 (2H, m), 1.57 (3H, s), 1.65 (3H, s), 1.7-2.1 (5H, m), 3.4-3.7 (2H, m), 5.08 (1H, m)

Preparation 32

To a suspension of sodium hydride (2.04 g) in N,N-dimethylformamide (50 ml) was added 4-hydroxypyridine (5 g) at room temperature. Octyl bromide (9.08 ml) was added thereto. The mixture was stirred for 2 hours at  $50^\circ\text{C}$ . The reaction mixture was added to a mixture of brine (100 ml), tetrahydrofuran (100 ml) and ethyl acetate (100 ml). The organic layer was separated and dried over magnesium sulfate. The magnesium sulfate was filtered off, and the filtrate was evaporated under reduced pressure to give 1-octyl-4-pyridone (14.7 g).

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6\text{Hz}$ ), 1.1-1.4 (10H, m), 1.4-1.8 (2H, m), 3.81 (2H, t,  $J=7\text{Hz}$ ), 6.05 (2H, d,  $J=8\text{Hz}$ ), 7.63 (2H, d,  $J=8\text{Hz}$ )

Preparation 33

To a solution of 1-octyl-4-pyridone (10.9 g) in pyridine (100 ml) was added phosphorous pentasulfide (8.65 g) at room temperature. The mixture was stirred for 3 hours at  $80^\circ\text{C}$ . The reaction mixture was added to a mixture of water (200 ml) and methylene chloride (200 ml). The organic layer was separated and dried over magnesium sulfate. The magnesium sulfate was filtered off, and the filtrate was evaporated under reduced pressure to give 1-octyl-1,4-dihydropyridine-4-thione (5.27 g).

IR (Neat) : 2910, 2850, 1620, 1460, 1110  $\text{cm}^{-1}$ 

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6\text{Hz}$ ), 1.1-1.4 (10H, m), 1.5-1.9 (2H, m), 3.95 (2H, t,  $J=7\text{Hz}$ ), 7.13 (2H, d,  $J=7\text{Hz}$ ), 7.60 (2H, d,  $J=7\text{Hz}$ )

The following compounds (Preparations 34 to 36) were obtained according to a similar manner to that of Preparation 1.

Preparation 34

## Methyl 2-(4-hydroxyphenyl)-2-methoxyacetate

IR (Nujol) : 3350, 1740, 1610, 1600, 1220, 1100  $\text{cm}^{-1}$ NMR (DMSO- $d_6$ ,  $\delta$ ) : 3.23 (3H, s), 3.60 (3H, s), 4.73 (1H, s), 6.72 (2H, d,  $J=8.9\text{Hz}$ ), 7.15 (2H, d,  $J=8.9\text{Hz}$ )5 EI-MS ( $m/z$ ) = 196 ( $M^+$ )Preparation 35

## D-Tyrosine methyl ester hydrochloride

10 IR (Nujol) : 3300, 1740, 1220  $\text{cm}^{-1}$ NMR (DMSO- $d_6$ ,  $\delta$ ) : 3.02 (2H, m), 3.67 (3H, s), 4.16 (1H, t,  $J=6.7\text{Hz}$ ), 6.72 (2H, d,  $J=8.4\text{Hz}$ ), 7.01 (2H, d,  $J=8.4\text{Hz}$ ), 8.58 (2H, s), 9.47 (1H, s)Preparation 36

15

## Methyl (4-hydroxyphenyl)glyoxylate

IR (Nujol) : 3380, 1730, 1700, 1600, 1580, 1220  $\text{cm}^{-1}$ NMR (DMSO- $d_6$ ,  $\delta$ ) : 3.91 (3H, s), 6.94 (2H, d,  $J=8.8\text{Hz}$ ), 7.83 (2H, d,  $J=8.8\text{Hz}$ ), 10.9 (1H, s)20 Preparation 37

N-(t-Butoxycarbonyl)-D-tyrosine methyl ester was obtained according to a similar manner to that of Preparation 2.

IR (Nujol) : 3360, 1700, 1680, 1290, 1270, 1250  $\text{cm}^{-1}$ 25 NMR (DMSO- $d_6$ ,  $\delta$ ) : 1.33 (9H, s), 2.73 (2H, m), 3.59 (3H, s), 4.05 (1H, m), 6.65 (2H, d,  $J=8.4\text{Hz}$ ), 7.00 (2H, d,  $J=8.4\text{Hz}$ ), 7.23 (1H, d,  $J=7.9\text{Hz}$ ), 9.23 (1H, s)Preparation 38

30 To a solution of L-tyrosine methyl ester hydrochloride (1 g) in water (1.5 ml) was added sodium bicarbonate (0.363 g) under ice-cooling and stirred for 10 minutes, and then acetonitrile (7 ml), 37% formaldehyde aqueous solution (0.637 ml) and sodium cyanoborohydride (0.182 g) was added thereto at  $-5^\circ\text{C}$ . The mixture was stirred for 2 hours at  $-5^\circ\text{C}$ . The resultant insoluble material was filtered off, and the filtrate was extracted with ethyl acetate. The organic layer was separated and dried over magnesium sulfate.

35 The magnesium sulfate was filtered off, and the filtrate was evaporated under reduced pressure to give N,N-dimethyl-L-tyrosine methyl ester (0.21 g).

IR (Nujol) : 1730, 1260, 1010  $\text{cm}^{-1}$ NMR (DMSO- $d_6$ ,  $\delta$ ) : 2.24 (6H, s), 2.72 (2H, m), 3.34 (1H, m), 3.53 (3H, s), 6.64 (2H, d,  $J=8.4\text{Hz}$ ), 6.97 (2H, d,  $J=8.4\text{Hz}$ ), 9.18 (1H, s)40 The following compounds (Preparations 39 to 44) were obtained according to a similar manner to that of Preparation 3.Preparation 39

## 45 Methyl 2-(4-octyloxyphenyl)acetate

IR (Neat) : 2910, 2850, 1730, 1240  $\text{cm}^{-1}$ NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.3\text{Hz}$ ), 1.2-1.5 (10H, m), 1.6-1.9 (2H, m), 3.58 (2H, s), 3.59 (3H, s), 3.92 (2H, t,  $J=6.4\text{Hz}$ ), 6.85 (2H, d,  $J=8.7\text{Hz}$ ), 7.15 (2H, d,  $J=8.7\text{Hz}$ )50 Preparation 40

## Ethyl 3-(4-octyloxyphenyl)propionate

IR (Neat) : 2920, 2850, 1730, 1240  $\text{cm}^{-1}$ 

55 NMR (DMSO- $d_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.7\text{Hz}$ ), 1.15 (3H, t,  $J=7.1\text{Hz}$ ), 1.2-1.5 (10H, m), 1.6-1.8 (2H, m), 2.55 (2H, t,  $J=7.2\text{Hz}$ ), 2.77 (2H, t,  $J=7.2\text{Hz}$ ), 3.90 (2H, t,  $J=6.4\text{Hz}$ ), 4.03 (2H, q,  $J=7.1\text{Hz}$ ), 6.81 (2H, d,  $J=8.6\text{Hz}$ ), 7.11 (2H, d,  $J=8.6\text{Hz}$ )

Preparation 41

## Methyl 2-(4-octyloxyphenyl)-2-methoxyacetate

IR (Neat) : 2910, 2850, 1740, 1600, 1240, 1100  $\text{cm}^{-1}$ NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.8\text{Hz}$ ), 1.2-1.5 (10H, m), 1.6-1.8 (2H, m), 3.26 (3H, s), 3.62 (3H, s), 3.94 (2H, t,  $J=6.4\text{Hz}$ ), 4.83 (1H, s), 6.91 (2H, d,  $J=8.7\text{Hz}$ ), 7.27 (2H, d,  $J=8.7\text{Hz}$ )EI-MS ( $m/z$ ) = 308 ( $M^+$ )Preparation 42O<sup>4</sup>-Octyl-N-(t-butoxycarbonyl)-D-tyrosine methyl esterIR (Nujol) : 3350, 1730, 1680, 1510, 1240, 1160  $\text{cm}^{-1}$ NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.7\text{Hz}$ ), 1.2-1.3 (10H, m), 1.68 (2H, m), 2.82 (2H, m), 3.60 (3H, s), 3.91 (2H, t,  $J=7.3\text{Hz}$ ), 4.08 (1H, m), 6.81 (2H, d,  $J=8.6\text{Hz}$ ), 7.12 (2H, d,  $J=8.6\text{Hz}$ ), 7.25 (1H, d,  $J=8.0\text{Hz}$ )Preparation 43O<sup>4</sup>-Octyl-N,N-dimethyl-L-tyrosine methyl esterIR (Neat) : 2930, 2860, 1730, 1250  $\text{cm}^{-1}$ NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.6\text{Hz}$ ), 1.26 (10H, m), 1.68 (2H, m), 2.80 (2H, m), 3.33 (6H, s), 3.37 (1H, m), 3.53 (3H, s), 3.89 (2H, t,  $J=6.4\text{Hz}$ ), 6.79 (2H, d,  $J=8.6\text{Hz}$ ), 7.08 (2H, d,  $J=8.6\text{Hz}$ )Preparation 44

## Methyl (4-octyloxyphenyl)glyoxylate

IR (Neat) : 2930, 2850, 1730, 1670, 1600, 1260, 1210, 1160  $\text{cm}^{-1}$ NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.3\text{Hz}$ ), 1.2-1.5 (10H, m), 1.6-1.9 (2H, m), 3.93 (3H, s), 4.10 (2H, t,  $J=6.5\text{Hz}$ ), 7.12 (2H, d,  $J=8.9\text{Hz}$ ), 7.92 (2H, d,  $J=8.9\text{Hz}$ )The following compounds (Preparations 45 to 51) were obtained according to a similar manner to that of Preparation 4.Preparation 45

## 4-(2-Butoxyethoxy)benzoic acid

IR (Nujol) : 1670, 1610, 1260  $\text{cm}^{-1}$ NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.87 (3H, t,  $J=7.2\text{Hz}$ ), 1.2-1.6 (4H, m), 3.45 (2H, t,  $J=6.4\text{Hz}$ ), 3.70 (2H, t,  $J=4.4\text{Hz}$ ), 4.16 (2H, t,  $J=4.4\text{Hz}$ ), 7.02 (2H, d,  $J=8.9\text{Hz}$ ), 7.88 (2H, d,  $J=8.9\text{Hz}$ ), 12.63 (1H, s)Preparation 46

## 2-(4-Octyloxyphenyl)acetic acid

IR (Nujol) : 1680, 1240, 820, 780  $\text{cm}^{-1}$ NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.8\text{Hz}$ ), 1.1-1.5 (10H, m), 1.6-1.8 (2H, m), 3.47 (2H, s), 3.92 (2H, t,  $J=6.4\text{Hz}$ ), 6.84 (2H, d,  $J=8.6\text{Hz}$ ), 7.14 (2H, d,  $J=8.6\text{Hz}$ )Preparation 47

## 3-(4-Octyloxyphenyl)propionic acid

IR (Nujol) : 1680, 1500, 1200  $\text{cm}^{-1}$ NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.3\text{Hz}$ ), 1.1-1.5 (10H, m), 1.6-1.8 (2H, m), 2.47 (2H, t,  $J=7.2\text{Hz}$ ), 2.74 (2H, t,  $J=7.2\text{Hz}$ ), 3.90 (2H, t,  $J=6.4\text{Hz}$ ), 6.81 (2H, d,  $J=8.6\text{Hz}$ ), 7.11 (2H, d,  $J=8.6\text{Hz}$ ), 12.10 (1H, br s)Preparation 48

## 2-(4-Octyloxyphenyl)-2-methoxyacetic acid

IR (Nujol) : 1760, 1720, 1600, 1500, 1240, 1180, 1100, 830  $\text{cm}^{-1}$   
 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.7\text{Hz}$ ), 1.2-1.5 (10H, m), 2.6-2.8 (2H, m), 3.26 (3H, s), 3.94 (2H, t,  $J=6.4\text{Hz}$ ), 4.67 (1H, s), 6.90 (2H, d,  $J=8.6\text{Hz}$ ), 7.27 (2H, d,  $J=8.6\text{Hz}$ )

#### 5 Preparation 49

O<sup>4</sup>-Octyl-N-(t-butoxycarbonyl)-D-tyrosine

IR (Nujol) : 3400-2900, 1700, 1500, 1240, 1160  $\text{cm}^{-1}$

10 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.859 (3H, t,  $J=6.8\text{Hz}$ ), 1.20-1.30 (10H, m), 1.32 (9H, s), 1.68 (2H, m), 2.67-2.95 (1H, m), 3.90 (2H, t,  $J=7\text{Hz}$ ), 4.01 (1H, m), 6.81 (2H, d,  $J=8.6\text{Hz}$ ), 7.02 (1H, d,  $J=8.3\text{Hz}$ ), 7.13 (2H, d,  $J=8.6\text{Hz}$ )

#### Preparation 50

15 O<sup>4</sup>-Octyl-N,N-dimethyl-L-tyrosine

IR (Neat) : 2940, 2860, 2600, 1620, 1240  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.6\text{Hz}$ ), 1.26 (10H, m), 1.68 (2H, m), 2.67 (6H, s), 2.8-3.6 (3H, m), 3.91 (2H, t,  $J=6.4\text{Hz}$ ), 6.85 (2H, d,  $J=8.5\text{Hz}$ ), 7.16 (2H, d,  $J=8.5\text{Hz}$ )

#### 20 Preparation 51

O<sup>4</sup>-Octyloxyphenylglyoxylic acid

IR (Neat) : 1730, 1670, 1600, 1260, 1160  $\text{cm}^{-1}$

25 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.8\text{Hz}$ ), 1.2-1.5 (10H, m), 1.65-1.85 (2H, m), 4.09 (2H, t,  $J=6.5\text{Hz}$ ), 7.12 (2H, d,  $J=8.9\text{Hz}$ ), 7.89 (2H, d,  $J=8.9\text{Hz}$ )

#### Preparation 52

30 N<sup>7</sup>-Octyl-N-(t-butoxycarbonyl)-L-histidine was obtained from N-(t-butoxycarbonyl)-L-histidine methyl ester according to similar manners to those of Preparations 3 and 4.

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.85 (3H, t,  $J=6.3\text{Hz}$ ), 1.23 (10H, m), 1.35 (9H, s), 2.83 (2H, m), 3.90 (2H, t,  $J=7\text{Hz}$ ), 4.0-4.2 (1H, m), 6.36 (1H, s), 7.02 (1H, d,  $J=8\text{Hz}$ ), 7.75 (1H, s)

The following compounds (Preparations 53 to 60) were obtained according to a similar manner to that of Preparation 11.

#### 35 Preparation 53

4-Octyloxyphthalic acid

IR (Neat) : 2930, 2860, 2500, 1700, 1600, 1260  $\text{cm}^{-1}$

40 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.8\text{Hz}$ ), 1.2-1.5 (10H, m), 1.5-1.8 (2H, m), 4.05 (2H, t,  $J=6.2\text{Hz}$ ), 7.03 (1H, d,  $J=2.6\text{Hz}$ ), 7.06 (1H, dd,  $J=8.4\text{Hz}$  and  $2.6\text{Hz}$ ), 7.72 (1H, d,  $J=8.4\text{Hz}$ )

#### Preparation 54

45 3-Methoxy-4-octyloxybenzoic acid

IR (Nujol) : 2600, 1680, 1600, 1270, 1230  $\text{cm}^{-1}$

50 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.8\text{Hz}$ ), 1.2-1.5 (10H, m), 1.6-1.8 (2H, m), 3.80 (3H, s), 4.01 (2H, t,  $J=6.5\text{Hz}$ ), 7.03 (1H, d,  $J=8.5\text{Hz}$ ), 7.44 (1H, d,  $J=1.9\text{Hz}$ ), 7.54 (1H, dd,  $J=8.5\text{Hz}$  and  $1.9\text{Hz}$ )

#### Preparation 55

4-(4-Octyloxyphenyl)benzoic acid

55 IR (Nujol) : 1670, 1600, 830, 770  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.87 (3H, t,  $J=6.7\text{Hz}$ ), 1.2-1.5 (10H, m), 1.6-1.8 (2H, m), 4.01 (2H, t,  $J=6.4\text{Hz}$ ), 7.04 (2H, d,  $J=8.8\text{Hz}$ ), 7.68 (2H, d,  $J=8.8\text{Hz}$ ), 7.75 (2H, d,  $J=8.5\text{Hz}$ ), 7.99 (2H, d,  $J=8.5\text{Hz}$ )

Preparation 56

## 6-(2-Ethylhexyloxy)-2-naphthoic acid

IR (Nujol) : 1660, 1610, 1280, 1200  $\text{cm}^{-1}$ 

5 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.88 (3H, t,  $J=7.3\text{Hz}$ ), 0.92 (3H, t,  $J=7.3\text{Hz}$ ), 1.2-1.6 (8H, m), 1.7-1.9 (1H, m), 4.01 (2H, d,  $J=5.7\text{Hz}$ ), 7.23 (1H, dd,  $J=8.9$  and  $2.4\text{Hz}$ ), 7.42 (1H, d,  $J=2.4\text{Hz}$ ), 7.86 (1H, d,  $J=8.7\text{Hz}$ ), 7.94 (1H, d,  $J=8.7\text{Hz}$ ), 8.01 (1H, d,  $J=8.9\text{Hz}$ ), 8.51 (1H, s), 12.9 (1H, s)

10 Preparation 57

## 6-(3,7-Dimethyl-6-octenyloxy)naphthoic acid

IR (Nujol) : 1660, 1610, 1290, 1200  $\text{cm}^{-1}$ 

15 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.95 (3H, d,  $J=6.1\text{Hz}$ ), 1.1-1.5 (2H, m), 1.57 (3H, s), 1.64 (3H, s), 1.6-2.1 (5H, m), 4.15 (2H, t,  $J=6.7\text{Hz}$ ), 5.10 (1H, t,  $J=7.1\text{Hz}$ ), 7.22 (1H, dd,  $J=8.9\text{Hz}$  and  $2.3\text{Hz}$ ), 7.42 (1H, d,  $J=2.3\text{Hz}$ ), 7.86 (1H, d,  $J=8.6\text{Hz}$ ), 7.94 (1H, d,  $J=8.6\text{Hz}$ ), 8.01 (1H, d,  $J=8.9\text{Hz}$ ), 8.52 (1H, s), 12.89 (1H, s)

Preparation 58

## 6-(3,7-Dimethyl-2,6-octadienyloxy)naphthoic acid

IR (Nujol) : 1660, 1620, 1210  $\text{cm}^{-1}$ 

20 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 1.57 (3H, s), 1.60 (3H, s), 1.76 (3H, s), 2.07 (4H, m), 4.70 (2H, d,  $J=6.5\text{Hz}$ ), 5.07 (1H, m), 5.51 (1H, t,  $J=6.5\text{Hz}$ ), 7.24 (1H, dd,  $J=8.9\text{Hz}$  and  $2.4\text{Hz}$ ), 7.41 (1H, d,  $J=2.4\text{Hz}$ ), 7.85 (1H, d,  $J=8.7\text{Hz}$ ), 7.94 (1H, d,  $J=8.7\text{Hz}$ ), 8.01 (1H, d,  $J=8.9\text{Hz}$ ), 8.52 (1H, s), 12.88 (1H, s)

Preparation 59

## (2E)-3(4-Octyloxyphenyl)acrylic acid

IR (Nujol) : 1660, 1600, 1240  $\text{cm}^{-1}$ 

30 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.7\text{Hz}$ ), 1.2-1.5 (10H, m), 1.6-1.8 (2H, m), 4.00 (2H, t,  $J=6.4\text{Hz}$ ), 6.36 (1H, d,  $J=16\text{Hz}$ ), 6.95 (2H, d,  $J=8.7\text{Hz}$ ), 7.54 (1H, d,  $J=16\text{Hz}$ ), 7.61 (2H, d,  $J=8.7\text{Hz}$ ), 12.20 (1H, br s)

35 Preparation 60

## Sodium 6-octyloxy-2-naphthalene sulfonate

IR (Nujol) : 1230, 1180, 860, 820  $\text{cm}^{-1}$ 

40 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6\text{Hz}$ ), 1.1-1.6 (10H, m), 4.06 (2H, t,  $J=5\text{Hz}$ ), 7.08 (1H, d,  $J=9\text{Hz}$ ), 7.21 (1H, s), 7.79 (1H, d,  $J=9\text{Hz}$ ), 8.00 (1H, s)

Preparation 61

45 To a solution of thionyl chloride (0.692 ml) and N,N-dimethylformamide (0.022 ml) was added sodium 6-octyloxy-2-naphthalenesulfonate (1 g) under ice-cooling and stirred for 1.5 hours at  $95^\circ\text{C}$ . The reaction mixture was evaporated under reduced pressure to give 6-octyloxy-2-naphthylsulfonyl chloride (1 g).

IR (Nujol) : 1610, 1260, 1160  $\text{cm}^{-1}$ 

50 NMR ( $\text{CDCl}_3$ ,  $\delta$ ) : 0.90 (3H, t,  $J=6.2\text{Hz}$ ), 1.2-1.7 (10H, m), 1.8-2.0 (2H, m), 4.12 (2H, t,  $J=6.5\text{Hz}$ ), 7.20 (1H, d,  $J=2.2\text{Hz}$ ), 7.32 (1H, dd,  $J=9.0\text{Hz}$  and  $2.2\text{Hz}$ ), 7.84-7.97 (3H, m), 8.49 (1H, s)

The following compounds (Preparations 62 to 71) were obtained according to a similar manner to that of Preparation 12.

55 Preparation 62

## 1-(4-Octylbenzoyl)-1H-benzotriazole-3-oxide

IR (Neat) : 2930, 2850, 1780, 1610, 1240, 990  $\text{cm}^{-1}$



Preparation 63

1-[4-(4-Octyloxyphenyl)benzoyl]-1H-benzotriazole-3-oxide  
 IR (Nujol) : 1770, 1600, 980  $\text{cm}^{-1}$

5

Preparation 64

1-[6-(2-Ethylhexyloxy)-2-naphthoyl]-1H-benzotriazole-3-oxide  
 IR (Nujol) : 1770, 1620, 1270, 1180  $\text{cm}^{-1}$   
 10 NMR ( $\text{CDCl}_3$ ,  $\delta$ ) : 0.93 (3H, t,  $J = 7.1\text{Hz}$ ), 0.98 (3H, t,  $J = 7.4\text{Hz}$ ), 1.3-1.7 (8H, m), 1.7-2.0 (1H, m), 4.03 (2H, d,  $J = 5.7\text{Hz}$ ), 7.22 (1H, d,  $J = 2.2\text{Hz}$ ), 7.29 (1H, dd,  $J = 8.9\text{Hz}$ ,  $2.2\text{Hz}$ ), 7.4-7.7 (3H, m), 7.87 (1H, d,  $J = 9.5\text{Hz}$ ), 7.92 (1H, d,  $J = 9.5\text{Hz}$ ), 8.1-8.2 (2H, m), 8.80 (1H, s)

15 Preparation 65

1-[6-(3,7-Dimethyl-6-octenyloxy)-2-naphthoyl]-1H-benzotriazole-3-oxide  
 IR (Neat) : 2900, 1770, 1620, 1180  $\text{cm}^{-1}$

20 Preparation 66

1-[6-{(E)-3,7-Dimethyl-2,6-octadienyloxy}-2-naphthoyl]-1H-benzotriazole-3-oxide  
 IR (Nujol) : 1770, 1620, 1270, 1180  $\text{cm}^{-1}$

25 Preparation 67

1-(2-Anthrylcarbonyl)-1H-benzotriazole-3-oxide  
 IR (Nujol) : 1780, 1200, 720, 740  $\text{cm}^{-1}$

30 Preparation 68

1-[2-(4-Octyloxyphenyl)acetyl]-1H-benzotriazole-3-oxide  
 IR (Nujol) : 1730, 1460, 1420, 1250, 1130  $\text{cm}^{-1}$

35 Preparation 69

1-[3-(4-Octyloxyphenyl)propionyl]-1H-benzotriazole-3-oxide  
 IR (Nujol) : 1730, 1420, 1340, 1240, 950  $\text{cm}^{-1}$

40 Preparation 70

1-1(E)-3-(4-Octyloxyphenyl)acryloyl]-1H-benzotriazole-3-oxide  
 IR (Nujol) : 1770, 1600, 1260, 1080  $\text{cm}^{-1}$

45 Preparation 71

1-(O<sup>4</sup>-Octyl-N,N-dimethyl-L-tyrosyl)-1H-benzotriazole-3-oxide  
 IR (Neat) : 2930, 2850, 1800, 1610  $\text{cm}^{-1}$

50 Preparation 72

To a suspension of lithium aluminum hydride (4.05 g) in tetrahydrofuran (475 ml) was added dropwise a solution of 4-octyloxybenzaldehyde (25 g) in tetrahydrofuran (25 ml) at 55 ~ 60 °C. The reaction mixture was stirred under reflux for 1 hour, and thereto was added sodium fluoride (35.84 g) and water (11.52 ml)  
 55 under ice-cooling. The mixture was stirred for 30 minutes, and filtrated. The filtrate was evaporated in vacuo to give 4-octyloxybenzyl alcohol (25.1 g) as crystals.

IR (Nujol) : 3200, 1605, 1510  $\text{cm}^{-1}$

NMR ( $\text{DMSO}-d_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J = 6.7\text{Hz}$ ), 1.26-1.38 (10H, m), 1.62-1.72 (2H, m), 3.92 (2H, t,

J = 6.5 Hz), 4.40 (2H, d, J = 5.7 Hz), 5.03 (1H, t, J = 5.7 Hz), 6.85 (2H, d, J = 8.6 Hz), 7.20 (2H, d, J = 8.6 Hz)

### Preparation 73

To a suspension of 4-octyloxybenzyl alcohol (25 g), N-hydroxyphthalimide (17.15 g) and triphenylphosphine (27.74 g) in tetrahydrofuran (250 ml) was added dropwise diethyl azodicarboxylate (18.4 g) under ice-cooling. The reaction mixture was stirred at room temperature for 2 hours, and evaporated in vacuo. The residue was purified by chromatography on silica gel to give N-(4-octyloxybenzyloxy)phthalimide (33.45 g) as crystals.

IR (Nujol) : 1780, 1725, 1605, 1580, 1505  $\text{cm}^{-1}$   
 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, m), 1.26 (10H, m), 1.70 (2H, m), 3.95 (2H, t, J = 6.5 Hz), 5.08 (2H, s), 6.93 (2H, d, J = 8.6 Hz), 7.40 (2H, d, J = 8.6 Hz), 7.85 (4H, s)

### Preparation 74

To a solution of N-(4-octyloxybenzyloxy)phthalimide (4.13 g) in tetrahydrofuran (16 ml) was added hydrazine-hydrate (0.53 ml) at room temperature. After the mixture was stirred at the same temperature for 1 hour, the precipitate was filtered off. To the filtrate was added water (6 ml) and 4-hydroxyphenylglyoxylic acid (1.5 g) at room temperature. The mixture was maintained at pH 4–4.5 with aqueous sodium bicarbonate solution for 2 hours, thereto was added ethyl acetate, and adjusted to pH 2 with 1N hydrochloric acid. The separated organic layer was washed with brine, and dried over magnesium sulfate. The organic solvent was evaporated in vacuo to give 2-(4-hydroxyphenyl)-2-(4-octyloxybenzyloxyimino)acetic acid (3.4 g).

IR (Nujol) : 3400, 1715, 1605, 1590, 1505  $\text{cm}^{-1}$   
 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, m), 1.25 (10H, m), 1.69 (2H, m), 3.94 (2H, t, J = 6.4 Hz), 5.07 (2H, s), 6.82 (2H, d, J = 8.7 Hz), 6.90 (2H, d, J = 8.6 Hz), 7.29 (2H, d, J = 8.6 Hz), 7.35 (2H, d, J = 8.7 Hz)

The following compounds (Preparations 75 and 76) were obtained according to a similar manner to that of Preparation 74.

### Preparation 75

2-Phenyl-2-(4-octyloxybenzyloxyimino)acetic acid

IR (Nujol) : 1720, 1610, 1585, 1515  $\text{cm}^{-1}$   
 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t, J = 6.7 Hz), 1.26 (10H, m), 1.69 (2H, m), 3.94 (2H, t, J = 6.5 Hz), 5.13 (2H, s), 6.91 (2H, d, J = 8.6 Hz), 7.22–7.49 (7H, m)

### Preparation 76

2-(4-Octyloxybenzyloxyimino)acetic acid

IR (Nujol) : 1700, 1670, 1600  $\text{cm}^{-1}$   
 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t, J = 6.2 Hz), 1.26 (10H, m), 1.70 (2H, m), 3.95 (2H, t, J = 6.5 Hz), 5.13 (2H, s), 6.91 (2H, d, J = 8.6 Hz), 7.29 (2H, d, J = 8.6 Hz), 7.56 (1H, s)

### Preparation 77

A solution of 4-octyloxyphenylglyoxylic acid (0.935 g) in a mixture of water (9 ml) and tetrahydrofuran (18 ml) and adjusted to pH 3.5–4 with 1N hydrochloric acid and methoxyamine hydrochloride (0.337 g) was added thereto at room temperature. The mixture was stirred for 2 hours at room temperature maintaining pH 3.5–4 with 1N hydrochloric acid. The reaction mixture was added to ethyl acetate. The organic layer was separated and dried over magnesium sulfate. The magnesium sulfate was filtered off, and the filtrate was evaporated under reduced pressure to give 2-(4-octyloxyphenyl)-2-methoxyiminoacetic acid (0.57 g).

IR (Nujol) : 1700, 1600, 1250, 1030  $\text{cm}^{-1}$   
 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t, J = 6.3 Hz), 1.2–1.5 (10H, m), 1.6–1.8 (2H, m), 3.89 (3H, s), 3.99 (2H, t, J = 6.4 Hz), 7.00 (2H, d, J = 8.9 Hz), 7.45 (2H, d, J = 8.9 Hz), 14.05 (1H, s)

### Preparation 78

To a mixture of 2,3,4,5,6-pentafluorobenzoic acid (1 g) and 2,2,3,3,4,4,5,5-octafluoropentanol (1.18 g) in N,N-dimethylformamide (5 ml) was added 62% sodium hydride (0.39 g) at room temperature. The mixture was stirred at the same temperature for 1 hour, and thereto was added a mixture of water and ethyl acetate. The separated organic layer was washed with water and brine, dried over magnesium sulfate, and evaporated in vacuo. The residue was purified by chromatography on silica gel to give 4-(2,2,3,3,4,4,5,5-octafluoropentyloxy)-2,3,5,6-tetrafluorobenzoic acid (923.0 mg).

IR (Nujol) : 1700, 1580  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 4.96 (2H, t,  $J = 14.2\text{Hz}$ ), 7.10 (1H, tt,  $J = 5.6\text{Hz}$  and  $50.2\text{Hz}$ )

#### 10 Preparation 79

4-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Pentadecafluorooctyloxy)-2,3,5,6-tetrafluorobenzoic acid

IR (Nujol) : 3400, 1640, 1560  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 4.95 (2H, t,  $J = 14.0\text{Hz}$ )

15 The following compounds (Preparations 80 to 90) were obtained according to a similar manner to that of Preparation 5.

#### Preparation 80

20 Succinimido 2-(4-hydroxyphenyl)-2-(4-octyloxybenzyloxyimino)acetate

IR (Nujol) : 1800, 1770, 1700, 1600  $\text{cm}^{-1}$

#### Preparation 81

25 Succinimido 2-phenyl-2-(4-octyloxybenzyloxyimino)acetate

IR (Nujol) : 1780, 1730, 1605  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, m), 1.26 (10H, m), 1.69 (2H, m), 2.90 (4H, m), 3.94 (2H, t,  $J = 6.4\text{Hz}$ ), 5.30 (2H, s), 6.91 (2H, d,  $J = 8.6\text{Hz}$ ), 7.25-7.56 (7H, m)

#### 30 Preparation 82

Succinimido 2-(4-Octyloxybenzyloxyimino)acetate

IR (Nujol) : 1760, 1725, 1600, 1580  $\text{cm}^{-1}$

35 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J = 6.7\text{Hz}$ ), 1.26 (10H, m), 1.70 (2H, m), 2.85 (4H, s), 3.96 (2H, m), 5.28 (2H, s), 6.91 (2H, d,  $J = 8.6\text{Hz}$ ), 7.33 (2H, d,  $J = 8.6\text{Hz}$ ), 8.12 (1H, s)

#### Preparation 83

Succinimido 4-(2,2,3,3,4,4,5,5-octafluoropentyloxy)-2,3,5,6-tetrafluorobenzoate

40 IR (Nujol) : 3500, 1770, 1740, 1640  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 2.90 (4H, s), 5.23 (2H, t,  $J = 13.8\text{Hz}$ ), 7.11 (1H, tt,  $J = 50.2\text{Hz}$  and  $5.6\text{Hz}$ )

#### Preparation 84

45 Succinimido 4-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctyloxy)-2,3,5,6-tetrafluorobenzoate

IR (Nujol) : 1735, 1620, 1600  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 2.90 (4H, s), 5.12 (2H, t,  $J = 13.8\text{Hz}$ )

#### Preparation 85

50 Succinimido 3-methoxy-4-octyloxybenzoate

IR (Nujol) : 1760, 1730, 1600, 1280, 1200, 880  $\text{cm}^{-1}$

55 NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J = 6.7\text{Hz}$ ), 1.2-1.5 (10H, m), 1.6-1.9 (2H, m), 2.88 (4H, s), 3.84 (3H, s), 4.09 (2H, t,  $J = 6.5\text{Hz}$ ), 7.19 (1H, d,  $J = 8.6\text{Hz}$ ), 7.49 (1H, d,  $J = 2.0\text{Hz}$ ), 7.73 (1H, dd,  $J = 8.6$  and  $2.0\text{Hz}$ )

#### Preparation 86

## Succinimido 4-(2-butoxyethoxy)benzoate

IR (Nujol) : 1730, 1600, 1250, 1060  $\text{cm}^{-1}$ NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.87 (3H, t,  $J=7.2\text{Hz}$ ), 1.2-1.6 (4H, m), 2.89 (4H, s), 3.46 (2H, t,  $J=6.3\text{Hz}$ ), 3.73 (2H, t,  $J=4.4\text{Hz}$ ), 4.25 (2H, t,  $J=4.4\text{Hz}$ ), 7.18 (2H, d,  $J=9.0\text{Hz}$ ), 8.04 (2H, d,  $J=9.0\text{Hz}$ )

5

Preparation 87

## Succinimido 2-(4-Octyloxyphenyl)-2-methoxyacetate

IR (Nujol) : 1810, 1740, 1610, 1250, 1210, 1100  $\text{cm}^{-1}$ NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.7\text{Hz}$ ), 1.2-1.5 (10H, m), 1.6-1.8 (2H, m), 2.80 (4H, s), 3.35 (3H, s), 3.97 (2H, t,  $J=6.4\text{Hz}$ ), 5.35 (1H, s), 6.96 (2H, d,  $J=8.7\text{Hz}$ ), 7.38 (2H, d,  $J=8.7\text{Hz}$ )

10

15 Preparation 88O<sup>4</sup>-Octyl-N-(t-butoxycarbonyl)-D-tyrosine succinimido esterIR (Nujol) : 3370, 1780, 1730, 1700, 1250, 1200  $\text{cm}^{-1}$ 20 Preparation 89

## Succinimido 2-(4-octyloxyphenyl)-2-methoxyiminoacetate

IR (Nujol) : 1800, 1780, 1730, 1600, 1250, 1180, 1130  $\text{cm}^{-1}$ NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.6\text{Hz}$ ), 1.2-1.5 (10H, m), 1.6-1.8 (2H, m), 2.89 (4H, s), 4.01 (3H, s), 4.03 (2H, t,  $J=6.4\text{Hz}$ ), 7.08 (2H, d,  $J=8.9\text{Hz}$ ), 7.68 (2H, d,  $J=8.9\text{Hz}$ )

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Preparation 90N<sup>7</sup>-Octyl-N-(t-butoxycarbonyl)-L-histidine succinimido esterIR (Neat) : 1810, 1780, 1730, 1500, 1360, 1200, 1160  $\text{cm}^{-1}$ 

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Preparation 914-Octyloxyphthalic anhydride was obtained from 4-octyloxyphthalic acid according to a similar manner to that of Preparation 5.

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IR (Neat) : 2910, 2850, 1840, 1760, 1640, 1610, 1290, 1260  $\text{cm}^{-1}$ NMR ( $\text{DMSO-d}_6$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.8\text{Hz}$ ), 1.2-1.5 (10H, m), 1.6-1.9 (2H, m), 4.19 (2H, t,  $J=6.5\text{Hz}$ ), 7.47 (1H, dd,  $J=8.4\text{Hz}$  and  $2.2\text{Hz}$ ), 7.57 (1H, d,  $J=2.2\text{Hz}$ ), 7.98 (1H, d,  $J=8.4\text{Hz}$ )

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Preparation 92N-Octyloxycarbonyloxysuccinimide was obtained according to a similar manner to that of Preparation 5.IR (Neat) : 2960, 2850, 1780, 1740, 1260, 1230  $\text{cm}^{-1}$ NMR ( $\text{CDCl}_3$ ,  $\delta$ ) : 0.89 (3H, t,  $J=6.7\text{Hz}$ ), 1.2-1.4 (10H, m), 1.6-1.8 (2H, m), 2.84 (4H, s), 4.32 (2H, t,  $J=6.7\text{Hz}$ )

45

Preparation 93

To a solution of octyl phenyl ether (1.53 g) in chloroform (6 ml) was added chlorosulfonic acid at 0° C. The mixture was stirred at room temperature for 30 minutes, then the mixture was poured into a mixture of water and tetrahydrofuran.

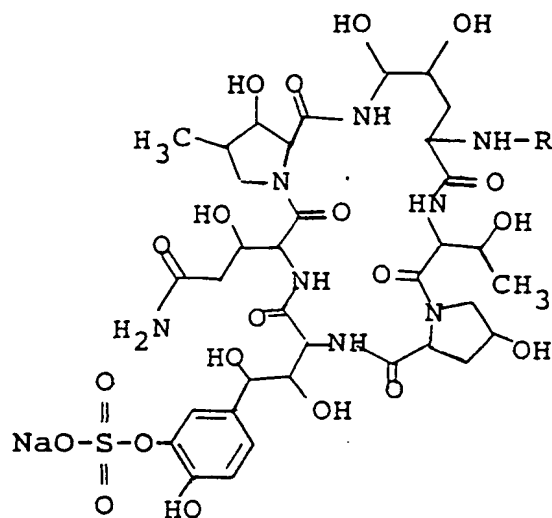
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The separated organic layer was washed with sodium chloride aqueous solution, dried over magnesium sulfate and then the solvent was evaporated in vacuo. The residue was subjected to a column chromatography on silica gel to give 4-octyloxyphenylsulfonyl chloride (1.25 g).

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IR (Nujol) : 1600, 1580, 1500, 1380, 1180  $\text{cm}^{-1}$ NMR ( $\text{CDCl}_3$ ,  $\delta$ ) : 0.89 (3H, t,  $J=6.6\text{Hz}$ ), 1.20-1.50 (10H, m), 1.80 (2H, m), 4.06 (2H, t,  $J=6.4\text{Hz}$ ), 7.03 (2H, d,  $J=9.0\text{Hz}$ ), 7.96 (2H, d,  $J=9.0\text{Hz}$ )

In the following, the structures of the compounds of Examples 13 to 53 are shown.



In the following formulae, <sup>t</sup>Bu means t-butyl, and p-TsOH means p-toluenesulfonic acid.

Example No.	Compound No.	R
13	FR139835	$-\text{COO}(\text{CH}_2)_7\text{CH}_3$
14	FR139537	$-\text{CO}-\text{C}_6\text{H}_4-\text{}^t\text{Bu}$
15	FR141145	$-\text{CO}-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)_3\text{CH}_3$
16	FR139538	$-\text{CO}-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_4-\text{C}_6\text{H}_5$

Example No.	Compound No.	R
17	FR140215	$\begin{array}{c} \text{---CO---} \text{C}_6\text{H}_3 \text{---O(CH}_2\text{)}_7\text{CH}_3 \\   \\ \text{COOH} \end{array}$
18	FR140216	$\begin{array}{c} \text{---CO---} \text{C}_6\text{H}_3 \text{---O(CH}_2\text{)}_7\text{CH}_3 \\   \\ \text{OCH}_3 \end{array}$
19	FR140727	$\begin{array}{c} \text{F} \quad \text{F} \\   \quad   \\ \text{---CO---} \text{C}_6\text{H}_2 \text{---OCH}_2(\text{CF}_2)_4\text{H} \\   \quad   \\ \text{F} \quad \text{F} \end{array}$
20	FR143301	$\begin{array}{c} \text{F} \quad \text{F} \\   \quad   \\ \text{---CO---} \text{C}_6\text{H}_2 \text{---OCH}_2(\text{CF}_2)_6\text{CF}_3 \\   \quad   \\ \text{F} \quad \text{F} \end{array}$
21	FR140495	$\text{---COCH}_2\text{---C}_6\text{H}_4\text{---C}_6\text{H}_5$
22	FR139503	$\begin{array}{c} \text{OCH}_3 \\   \\ \text{---COCH---} \text{C}_6\text{H}_4 \text{---O(CH}_2\text{)}_7\text{CH}_3 \end{array}$
23	FR139500	$\begin{array}{c} \text{NHCOO}^t\text{Bu} \\   \\ \text{---COCHCH}_2\text{---C}_6\text{H}_4 \text{---O(CH}_2\text{)}_7\text{CH}_3 \\ \text{(D)} \end{array}$
24	FR139501	$\begin{array}{c} \text{NHCOO}^t\text{Bu} \\   \\ \text{---CO---CH---} \text{C}_6\text{H}_4\text{---C}_6\text{H}_5 \\ \text{(L)} \end{array}$

Example No.	Compound No.	R
25	FR139502	$\begin{array}{c} \text{NHCOO}^t\text{Bu} \\   \\ -\text{COCHCH}_2 \text{---} \text{C}_5\text{H}_4\text{N} \text{---} \text{N}-(\text{CH}_2)_7\text{CH}_3 \\ \text{(L)} \end{array}$
26	FR138959	$\begin{array}{c} \text{OCH}_3 \\   \\ \text{N} \\    \\ -\text{CO}-\text{C}-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3 \end{array}$
27	FR140291	$\begin{array}{c} \text{O}-\text{CH}_2-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3 \\   \\ \text{N} \\    \\ -\text{CO}-\text{C}-\text{C}_6\text{H}_4-\text{OH} \end{array}$
28	FR141580	$\begin{array}{c} \text{O}-\text{CH}_2-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3 \\   \\ \text{N} \\    \\ -\text{CO}-\text{C}-\text{C}_6\text{H}_5 \end{array}$
29	FR141579	$\begin{array}{c} \text{O}-\text{CH}_2-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3 \\   \\ \text{N} \\    \\ -\text{CO}-\text{CH} \end{array}$
30	FR141146	$\text{CH}_3\text{C}(=\text{O})\text{CH}=\text{C}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)_2$
31	FR140731	$-\text{CO}-\text{C}_6\text{H}_4-(\text{CH}_2)_7\text{CH}_3$
32	FR140217	$-\text{CO}-\text{C}_6\text{H}_4-\text{O}-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3$

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Example No.	Compound No.	R
43	FR143020	$-\text{COCH}_2-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3$
44	FR143021	$-\text{CO}(\text{CH}_2)_2-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3$
45	FR141315	$-\text{CO}-\text{CH}=\text{CH}-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3$
46	FR140105	$\begin{array}{c} \text{N}(\text{CH}_3)_2 \\   \\ -\text{CO}-\text{CHCH}_2-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3 \end{array}$
47	FR141564	$-\text{SO}_2-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3$
48	FR143170	$-\text{SO}_2-\text{C}_6\text{H}_4-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3$
49	FR138912	$\begin{array}{c} \text{NH}_2 \cdot \text{p-TsOH} \\   \\ -\text{CO}-\text{CHCH}_2-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3 \\ (\text{L}) \end{array}$
50	FR138960	$\begin{array}{c} \text{Br}^- \\   \\ -\text{COCH}_2\text{S}-\text{C}_6\text{H}_4-\text{N}^+(\text{CH}_2)_7\text{CH}_3 \end{array}$
51	FR138727	$\begin{array}{c} \text{NH}_2 \\   \\ -\text{COCH}-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3 \\ (\text{D}) \end{array}$

Example No.	Compound No.	R
52	FR138912	$\begin{array}{c} \text{NH}_2 \\   \\ -\text{CO}-\text{CHCH}_2-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_7\text{CH}_3 \\ (\text{L}) \end{array}$
53	FR138960	$-\text{COCH}_2\text{S}-\text{C}_6\text{H}_4-\text{N}^+(\text{CH}_2)_7\text{CH}_3 \text{ Br}^-$

Example 13

FR139835 substance was obtained by reacting FR133303 substance with N-octyloxycarbonyloxysuccinimide according to a similar manner to that of Example 3.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

FAB-MS  $e/z$  = 1137 (M + Na)

Example 14

FR139537 substance was obtained by reacting FR133303 substance with succinimido 4-t-butylbenzoate according to a similar manner to that of Example 3.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

NMR ( $\text{D}_2\text{O}$ ,  $\delta$ ) : 1.05 (3H, d, J=6.9Hz), 1.15 (3H, d, J=5.9Hz), 1.33 (9H, s), 2.0-2.3 (3H, m), 2.4-2.6 (3H, m), 2.7-2.9 (1H, m), 3.4-3.6 (1H, m), 3.8-4.9 (12H, m), 5.07 (2H, m), 5.40 (1H, d, J=3Hz), 7.06 (1H, d, J=8.2Hz), 7.08 (1H, dd, J=8.2Hz and 2Hz), 7.27 (1H, d, J=2Hz), 7.60 (1H, d, J=8.6Hz), 7.75 (1H, d, J=8.6Hz)

Example 15

FR141145 substance was obtained by reacting FR133303 substance with succinimido 4-(2-butoxyethoxy)benzoate according to a similar manner to that of Example 3.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

NMR ( $\text{DMSO}-d_6$ , +  $\text{D}_2\text{O}$ ,  $\delta$ ) : 0.88 (3H, t, J=7.3Hz), 0.96 (3H, d, J=6.7Hz), 1.04 (3H, d, J=5.7Hz), 1.2-1.6 (4H, m), 1.7-2.0 (3H, m), 2.1-2.65 (4H, m), 3.16 (1H, m), 3.7-4.5 (20H, m), 4.78 (1H, d, J=3Hz), 4.86 (1H, d, J=3.8Hz), 5.02 (1H, d, J=3Hz), 6.74 (1H, d, J=8.2Hz), 6.79 (1H, d, J=8.2Hz), 7.00 (2H, d, J=8.9Hz), 7.06 (1H, s), 7.87 (2H, d, J=8.9Hz)

FAB-MS  $e/z$  = 1201 (M + Na)

Example 16

FR139538 substance was obtained by reacting FR133303 substance with succinimido 4-(4-phenylbutoxy)benzoate according to a similar manner to that of Example 3.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

FAB-MS  $e/z$  = 1233 (M + Na)

Example 17

FR140215 substance was obtained by reacting FR133303 substance with 4-octyloxyphthalic anhydride according to a similar manner to that of Example 3.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

FAB-MS  $m/z$  = 1257 (M + Na)

5

#### Example 18

FR140216 substance was obtained by reacting FR133303 substance with succinimido 3-methoxy-4-octyloxybenzoate according to a similar manner to that of Example 3.

10 IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

FAB-MS  $m/z$  = 1243 (M + Na)

#### Example 19

15 FR140727 substance was obtained by reacting FR133303 substance with succinimido 4-(2,2,3,3,4,4,5,5-octafluoropentyloxy)-2,3,5,6-tetrafluorobenzoate according to a similar manner to that of Example 3.

IR (Nujol) : 3300, 1630  $\text{cm}^{-1}$

FAB-MS  $m/z$  : 1387 (M + Na)

#### 20 Example 20

FR143301 substance was obtained by reacting FR133303 substance with succinimido 4-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctyloxy)-2,3,5,6-tetrafluorobenzoate according to a similar manner to that of Example 3.

25 IR (Nujol) : 3300, 1630  $\text{cm}^{-1}$

FAB-MS  $m/z$  = 1534 (M<sup>+</sup>)

#### Example 21

30 FR140495 substance was obtained by reacting FR133303 substance with succinimido 2-(4-biphenyl)-acetate according to a similar manner to that of Example 3.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

35 NMR ( $\text{CD}_3\text{OD}$ ,  $\delta$ ) : 1.0-1.1 (6H, m), 1.9-2.2 (3H, m), 2.3-2.6 (3H, m), 2.7-2.85 (1H, m), 3.35 (1H, m), 3.58 (2H, s), 3.65-4.7 (13H, m), 4.93 (1H, d, J=3Hz), 5.04 (1H, d, J=3.8Hz), 5.25 (1H, d, J=3Hz), 6.85 (1H, d, J=8.3Hz), 7.01 (1H, dd, J=8.3Hz and 2Hz), 7.3-7.6 (10H, m)

#### Example 22

40 FR139503 substance was obtained by reacting FR133303 substance with succinimido 2-(4-octyloxyphenyl)-2-methoxyacetate according to a similar manner to that of Example 3.

IR (Nujol) : 3330, 1620  $\text{cm}^{-1}$

FAB-MS  $m/z$  = 1257 (M + Na)

#### 45 Example 23

FR139500 substance was obtained by reacting FR133303 substance with O<sup>4</sup>-octyl-N-(t-butoxycarbonyl)-D-tyrosine succinimido ester according to a similar manner to that of Example 3.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

50 NMR ( $\text{CD}_3\text{OD}$ ,  $\delta$ ) : 0.90 (3H, t, J=6.8Hz), 1.06 (3H, d, J=6.8Hz), 1.17 (3H, d, J=6.7Hz), 1.20-1.30 (10H, m), 1.35 (9H, s), 1.74 (2H, m), 1.9-2.1 (3H, m), 2.45 (3N, m), 2.76 (1H, m), 3.0-3.1 (1H, m), 3.37 (1H, m), 3.7-4.6 (18H, m), 4.94 (1H, d, J=3Hz), 5.01 (1H, d, J=3.8Hz), 5.25 (1H, d, J=3Hz), 6.79 (2H, d, J=8.5Hz), 6.83 (1H, d, J=8.3Hz), 7.03 (1H, dd, J=8.3Hz and 2Hz), 7.12 (2H, d, J=8.5Hz), 7.31 (1H, d, J=2Hz)

55

#### Example 24

FR139501 substance was obtained by reacting FR133303 substance with N-(t-butoxycarbonyl)-L-2-(2-

naphthyl)glycine succinimido ester according to a similar manner to that of Example 3.  
IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

#### Example 25

FR139502 substance was obtained by reacting FR133303 substance with N<sup>r</sup>-octyl-N-(t-butoxycarbonyl)-L-histidine succinimido ester according to a similar manner to that of Example 3.  
IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$   
FAB-MS  $e/z$  = 1330 (M + Na)

#### Example 26

FR138959 substance was obtained by reacting FR133303 substance with succinimido 2-(4-octyloxyphenyl)-2-methoxyiminoacetate according to a similar manner to that of Example 3.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

NMR ( $\text{CD}_3\text{OD}$ ,  $\delta$ ) : 0.91 (3H, t, J=6.6Hz), 1.06 (3H, d, J=6.8Hz), 1.25 (3H, d, J=6.3Hz), 1.25-1.6 (10H, m), 1.65-1.9 (2H, m), 1.9-2.2 (3H, m), 2.3-2.65 (3H, m), 1.75-1.9 (1H, m), 3.3-3.5 (1H, m), 3.95 (3H, s), 3.7-4.75 (16H, m), 5.03 (1H, d, J=3.0Hz), 5.11 (1H, d, J=3.7Hz), 5.46 (1H, d, J=2.7Hz), 6.86 (1H, d, J=8.2Hz), 6.89 (2H, d, J=8.9Hz), 7.01 (1H, dd, J=8.2Hz and 2Hz), 7.31 (1H, d, J=2Hz), 7.54 (2H, d, J=8.9Hz)

FAB-MS  $e/z$  = 1270 (M + Na)

#### Example 27

FR140291 substance was obtained by reacting FR133303 substance with succinimido 2-(4-hydroxyphenyl)-2-(4-octyloxybenzyloxyimino)acetate according to a similar manner to that of Example 3.

IR (Nujol) : 3250, 1650, 1620  $\text{cm}^{-1}$

FAB-MS  $e/z$  = 1363 (M + Na)

#### Example 28

FR141580 substance was obtained by reacting FR133303 substance with succinimido 2-phenyl-2-(4-octyloxybenzyloxyimino)acetate according to a similar manner to that of Example 3.

IR (Nujol) : 3300, 1646  $\text{cm}^{-1}$

FAB-MS  $e/z$  = 1346 (M + Na)

Example 29 FR141579 substance was obtained by reacting FR133303 substance with succinimido 2-(4-octyloxybenzyloxyimino)acetate according to a similar manner to that of Example 3.

IR (Nujol) : 3250, 1650  $\text{cm}^{-1}$

FAB-MS  $e/z$  = 1270 (M + Na)

#### Example 30

FR141146 substance was obtained by reacting FR133303 substance with 1-[(2E,6E)-3,7,11-trimethyl-2,6,10-dodecatrienoyl]-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620, 1040  $\text{cm}^{-1}$

NMR ( $\text{CD}_3\text{OD}$ ,  $\delta$ ) : 1.06 (3H, d, J=6.8Hz), 1.19 (3H, d, J=5.9Hz), 1.60 (3H, s), 1.62 (3H, s), 1.66 (3H, s), 1.9-2.2 (11H, m), 2.05 (3H, s), 2.3-2.6 (3H, m), 2.7-2.9 (1H, m), 3.35 (1H, m), 3.7-5.0 (14H, m), 5.08 (4H, m), 5.27 (1H, d, J=2.8Hz), 5.77 (1H, s), 6.86 (1H, d, J=8.3Hz), 7.04 (1H, dd, J=8.3Hz and 1.9Hz), 7.32 (1H, d, J=1.9Hz)

#### Example 31

FR140731 substance was obtained by reacting FR133303 substance with 1-(4-octylbenzoyl)-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620, 1040  $\text{cm}^{-1}$

NMR ( $\text{CD}_3\text{OD}$ ,  $\delta$ ) : 0.86 (3H, t, J=6.8Hz), 1.06 (3H, d, J=6.8Hz), 1.21 (3H, d, J=5.8Hz), 1.25-1.45 (10H, m), 1.55-1.75 (2H, m), 1.9-2.25 (3H, m), 2.35-2.6 (3H, m), 2.65 (2H, t,

J=7.5Hz), 2.81 (1H, m), 3.32 (1H, m), 3.7-4.8 (14H, m), 4.98 (1H, d, J=3Hz), 5.09 (1H, d, J=3.9Hz), 5.31 (1H, d, J=3Hz), 6.86 (1H, d, J=8.3Hz), 7.03 (1H, dd, J=8.3Hz and 2Hz), 7.24 (2H, d, J=8.2Hz), 7.33 (1H, d, J=2Hz), 7.74 (2H, d, J=8.2Hz)

5 FAB-MS  $e/z$  = 1197 (M + Na)

#### Example 32

FR140217 substance was obtained by reacting FR133303 substance with 1-[4-(4-octyloxy)phenoxy]-benzoyl-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

FAB-MS  $e/z$  = 1305 (M + Na)

#### Example 33

FR142472 substance was obtained by reacting FR133303 substance with 1-[4-(4-octyloxyphenyl)-benzoyl]-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

20 NMR ( $\text{CD}_3\text{OD}$ ,  $\delta$ ) : 0.88 (3H, t, J=6.7Hz), 1.06 (3H, d, J=6.8Hz), 1.23 (3H, d, J=6.1Hz), 1.3-1.6 (10H, m), 1.8-1.9 (2H, m), 1.9-2.3 (3H, m), 2.3-2.7 (3H, m), 2.9-3.0 (1H, m), 3.39 (1H, m), 3.7-4.7 (16H, m), 4.99 (1H, d, J=3.0Hz), 5.10 (1H, d, J=3.7Hz), 5.35 (1H, d, J=2.7Hz), 6.87 (1H, d, J=8.3Hz), 6.99 (2H, d, J=8.8Hz), 7.04 (1H, dd, J=8.3Hz and 1.9Hz), 7.33 (1H, d, J=1.9Hz), 7.58 (2H, d, J=8.8Hz), 7.62 (2H, d, J=8.4Hz), 7.87 (2H, d, J=8.4Hz)

25 FAB-MS  $e/z$  = 1289 (M + Na)

#### Example 34

FR140496 substance was obtained by reacting FR133303 substance with 1-(6-butoxy-2-naphthoyl)-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

FAB-MS  $e/z$  = 1207 (M + Na)

#### Example 35

35 FR140497 substance was obtained by reacting FR133303 substance with 1-(6-hexyloxy-2-naphthoyl)-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

40 NMR ( $\text{DMSO}-d_6$  +  $\text{D}_2\text{O}$ ,  $\delta$ ) : 0.89 (3H, t, J=6.6Hz), 0.97 (3H, d, J=6.9Hz), 1.08 (3H, d, J=5.9Hz), 1.2-1.6 (6H, m), 1.7-2.1 (5H, m), 2.1-2.5 (3H, m), 2.5-2.7 (1H, m), 3.19 (1H, m), 3.73 (2H, m), 3.8-4.5 (12H, m), 4.80 (1H, d, J=3Hz), 4.88 (1H, d, J=3.8Hz), 5.08 (1H, d, J=3Hz), 6.74 (1H, d, J=8.2Hz), 6.80 (1H, dd, J=8.2Hz and 2Hz), 7.08 (1H, d, J=2Hz), 7.26 (1H, dd, J=8.9Hz and 2.4Hz), 7.39 (1H, d, J=2.4Hz), 7.85 (1H, d, J=8.7Hz), 7.89 (1H, d, J=8.7Hz), 7.93 (1H, d, J=8.9Hz), 8.44 (1H, s)

45 FAB-MS  $e/z$  = 1236 (M + Na)

#### Example 36

50 FR143483 substance was obtained by reacting FR133303 substance with 1-[6-(2-ethylhexyloxy)-2-naphthoyl]-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3250, 1620  $\text{cm}^{-1}$

55 NMR ( $\text{CD}_3\text{OD}$ ,  $\delta$ ) : 0.93 (3H, t, J=7.4Hz), 0.98 (3H, t, J=7.4Hz), 1.06 (3H, d, J=6.8Hz), 1.24 (3H, d, J=6.0Hz), 1.3-1.7 (8H, m), 1.7-1.9 (1H, m), 1.9-2.3 (3H, m), 2.3-2.7 (3H, m), 2.8-3.0 (1H, m), 3.39 (1H, m), 3.7-4.7 (16H, m), 5.00 (1H, d, J=4.4Hz), 5.11 (1H, d, J=3.7Hz), 5.37 (1H, d, J=2.6Hz), 6.87 (1H, d, J=8.3Hz), 7.04 (1H, dd, J=8.3Hz and 2Hz), 7.17 (1H, dd, J=8.9Hz and 1.9Hz), 7.22 (1H, d, J=2Hz), 7.33 (1H, d, J=1.9Hz), 7.7-7.9 (3H, m), 8.29 (1H, s) -

FAB-MS  $m/z$  = 1263 ( $M + Na$ )

#### Example 37

FR140728 substance was obtained by reacting FR133303 substance with 1-(6-decyloxy-2-naphthoyl)-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620  $cm^{-1}$

NMR ( $DMSO-d_6 + D_2O, \delta$ ) : 0.86 (3H, t,  $J=6.6Hz$ ), 0.97 (3H, d,  $J=6.7Hz$ ), 1.07 (3H, d,  $J=5.9Hz$ ), 1.2-1.6 (14H, m), 1.7-2.1 (5H, m), 2.1-2.5 (3H, m), 2.5-2.7 (1H, m), 3.19 (1H, m), 3.45 (1H, m), 3.73 (2H, m), 3.9-4.5 (12H, m), 4.79 (1H, d,  $J=3Hz$ ), 4.87 (1H, d,  $J=3.8Hz$ ), 5.07 (1H, d,  $J=3Hz$ ), 6.74 (1H, d,  $J=8.2Hz$ ), 6.79 (1H, dd,  $J=8.1Hz$  and  $2Hz$ ), 7.06 (1H, d,  $J=2Hz$ ), 7.23 (1H, dd,  $J=8.9Hz$  and  $2.4Hz$ ), 7.38 (1H, d,  $J=2.4Hz$ ), 7.85 (1H, d,  $J=8.7Hz$ ), 7.89 (1H, d,  $J=8.7Hz$ ), 7.93 (1H, d,  $J=8.9Hz$ ), 8.45 (1H, s)

FAB-MS  $m/z$  = 1291 ( $M + Na$ )

#### Example 38

FR142172 substance was obtained by reacting FR133303 substance with 1-[6-(3,7-dimethyloctyloxy)-2-naphthoyl]-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1610  $cm^{-1}$

NMR ( $DMSO-d_6 + D_2O, \delta$ ) : 0.85 (6H, d,  $J=6.6Hz$ ), 0.95 (3H, d,  $J=5.9Hz$ ), 0.97 (3H, d,  $J=6.7Hz$ ), 1.08 (3H, d,  $J=5.9Hz$ ), 1.1-1.4 (6H, m), 1.4-2.1 (7H, m), 2.1-2.5 (3H, m), 2.5-2.7 (1H, m), 3.19 (1H, m), 3.74 (2H, m), 3.9-4.6 (12H, m), 4.81 (1H, d,  $J=3Hz$ ), 4.87 (1H, d,  $J=3.8Hz$ ), 5.07 (1H, d,  $J=3Hz$ ), 6.74 (1H, d,  $J=8.2Hz$ ), 6.83 (1H, dd,  $J=8.1Hz$  and  $2Hz$ ), 7.06 (1H, d,  $J=2Hz$ ), 7.23 (1H, dd,  $J=8.9Hz$  and  $2.4Hz$ ), 7.40 (1H, d,  $J=2.4Hz$ ), 7.85 (1H, d,  $J=8.7Hz$ ), 7.89 (1H, d,  $J=8.7Hz$ ), 7.93 (1H, d,  $J=8.9Hz$ ), 8.45 (1H, s)

FAB-MS  $m/z$  = 1291 ( $M + Na$ )

#### Example 39

FR143326 substance was obtained by reacting FR133303 substance with 1-[6-(3,7-dimethyl-6-octenyloxy)-2-naphthoyl]-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620, 1260, 1040  $cm^{-1}$

NMR ( $CD_3OD, \delta$ ) : 1.00 (3H, d,  $J=6.2Hz$ ), 1.06 (3H, d,  $J=6.8Hz$ ), 1.25 (3H, d,  $J=5.9Hz$ ), 1.2-1.6 (2H, m), 1.61 (3H, s), 1.67 (3H, s), 1.63-2.3 (8H, m), 2.3-2.7 (3H, m), 2.8-3.0 (1H, m), 3.39 (1H, m), 3.7-4.8 (16H, m), 5.00 (1H, d,  $J=5.1Hz$ ), 5.08-5.2 (2H, m), 5.37 (1H, d,  $J=2.5Hz$ ), 6.87 (1H, d,  $J=8.3Hz$ ), 7.04 (1H, d,  $J=8.3Hz$ ), 7.15 (1H, d,  $J=8.9Hz$ ), 7.21 (1H, s), 7.33 (1H, s), 7.71 (1H, d,  $J=8.7Hz$ ), 7.77-7.85 (2H, m), 8.28 (1H, s)

#### Example 40

FR142390 substance was obtained by reacting FR133303 substance with 1-[6- $\{(E)-3,7$ -dimethyl-2,6-octadienyloxy}-2-naphthoyl]-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620  $cm^{-1}$

NMR ( $DMSO-d_6 + D_2O, \delta$ ) : 0.97 (3H, d,  $J=6.7Hz$ ), 1.07 (3H, d,  $J=6.0Hz$ ), 1.57 (3H, s), 1.61 (3H, s), 1.76 (3H, s), 1.8-2.5 (9H, m), 2.5-2.7 (1H, m), 3.19 (1H, m), 3.45 (1H, m), 3.73 (2H, m), 3.9-4.6 (11H, m), 4.70 (2H, d,  $J=6.5Hz$ ), 4.80 (1H, d,  $J=3Hz$ ), 4.87 (1H, d,  $J=3.8Hz$ ), 5.07 (2H, m), 5.51 (1H, t,  $J=6.5Hz$ ), 6.74 (1H, d,  $J=8.3Hz$ ), 6.83 (1H, dd,  $J=8.3Hz$  and  $2Hz$ ), 7.07 (1H, d,  $J=2Hz$ ), 7.24 (1H, dd,  $J=8.9Hz$  and  $2.4Hz$ ), 7.40 (1H, d,  $J=2.4Hz$ ), 7.8-8.0 (3H, m), 8.45 (1H, s)

FAB-MS  $m/z$  = 1287 ( $M + Na$ )

#### Example 41

FR140729 substance was obtained by reacting FR133303 substance with 1-(6-dodecyloxy-2-naphthoyl)-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1610  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$  +  $\text{D}_2\text{O}$ ,  $\delta$ ) : 0.85 (3H, t,  $J=6.6\text{Hz}$ ), 0.97 (3H, d,  $J=6.7\text{Hz}$ ), 1.07 (3H, d,  $J=5.9\text{Hz}$ ), 1.2-1.6 (18H, m), 1.7-2.1 (5H, m), 2.1-2.5 (3H, m), 2.5-2.7 (1H, m), 3.19 (1H, m), 3.45 (1H, m), 3.73 (2H, m), 3.9-4.5 (12H, m), 4.79 (1H, d,  $J=3\text{Hz}$ ), 4.87 (1H, d,  $J=3.8\text{Hz}$ ), 5.07 (1H, d,  $J=3\text{Hz}$ ), 6.74 (1H, d,  $J=8.1\text{Hz}$ ), 6.78 (1H, dd,  $J=8.1\text{Hz}$  and  $2\text{Hz}$ ), 7.06 (1H, d,  $J=2\text{Hz}$ ), 7.23 (1H, dd,  $J=8.9\text{Hz}$  and  $2.4\text{Hz}$ ), 7.38 (1H, d,  $J=2.4\text{Hz}$ ), 7.85 (1H, d,  $J=8.7\text{Hz}$ ), 7.89 (1H, d,  $J=8.7\text{Hz}$ ), 7.93 (1H, d,  $J=8.9\text{Hz}$ ), 8.44 (1H, s)

FAB-MS  $m/z$  = 1320 ( $M + \text{Na}$ )

#### Example 42

FR140730 substance was obtained by reacting FR133303 substance with 1-(2-anthrylcarbonyl)-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

FAB-MS  $m/z$  = 1185 ( $M + \text{Na}$ )

#### Example 43

FR143020 substance was obtained by reacting FR133303 substance with 1-[2-(4-octyloxyphenyl)-acetyl]-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

NMR ( $\text{CD}_3\text{OD}$ ,  $\delta$ ) : 0.87 (3H, t,  $J=6.8\text{Hz}$ ), 1.0-1.2 (6H, m), 1.2-1.6 (10H, m), 1.6-1.85 (2H, m), 1.85-2.1 (3H, m), 2.3-2.6 (3H, m), 2.7-2.85 (1H, m), 3.32 (1H, m), 3.46 (2H, s), 3.7-4.7 (16H, m), 5.04 (1H, d,  $J=3.7\text{Hz}$ ), 5.23 (1H, d,  $J=2.7\text{Hz}$ ), 6.75-6.9 (3H, m), 7.01 (1H, d,  $J=8.3\text{Hz}$ ), 7.15 (2H, d,  $J=8.5\text{Hz}$ ), 7.30 (1H, s)

FAB-MS  $m/z$  = 1227 ( $M + \text{Na}$ )

#### Example 44

FR143021 substance was obtained by reacting FR133303 substance with 1-[3-(4-octyloxyphenyl)-propionyl]-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

FAB-MS  $m/z$  = 1241 ( $M + \text{Na}$ )

#### Example 45

FR141315 substance was obtained by reacting FR133303 substance with 1-[(E)-3-(4-octyloxyphenyl)-acryloyl]-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$  +  $\text{D}_2\text{O}$ ,  $\delta$ ) : 0.86 (3H, t,  $J=6.7\text{Hz}$ ), 0.97 (3H, d,  $J=6.7\text{Hz}$ ), 1.04 (3H, d,  $J=5.4\text{Hz}$ ), 1.2-1.5 (10H, m), 1.6-2.0 (5H, m), 2.1-2.5 (3H, m), 2.5-2.6 (1H, m), 3.17 (1H, m), 3.3-4.5 (15H, m), 4.79 (1H, d,  $J=3\text{Hz}$ ), 4.86 (1H, d,  $J=3.8\text{Hz}$ ), 5.01 (1H, d,  $J=3\text{Hz}$ ), 6.57 (1H, d,  $J=15.8\text{Hz}$ ), 6.74 (1H, d,  $J=8.2\text{Hz}$ ), 6.82 (1H, d,  $J=8.2\text{Hz}$ ), 6.97 (2H, d,  $J=8.8\text{Hz}$ ), 7.09 (1H, s), 7.34 (1H, d,  $J=15.8\text{Hz}$ ), 7.52 (2H, d,  $J=8.8\text{Hz}$ )

FAB-MS  $m/z$  = 1239 ( $M + \text{Na}$ )

#### Example 46

FR140105 substance was obtained by reacting FR133303 substance with 1-(O<sup>4</sup>-octyl-N,N-dimethyl-L-tyrosyl)-1H-benzotriazole-3-oxide according to a similar manner to that of Example 12.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

NMR ( $\text{CD}_3\text{OD}$ ,  $\delta$ ) : 0.91 (3H, t,  $J=6.8\text{Hz}$ ), 1.06 (3H, d,  $J=6.8\text{Hz}$ ), 1.12 (3H, d,  $J=6.1\text{Hz}$ ), 1.33 (10H, m), 1.74 (2H, m), 1.98 (3H, m), 2.40 (6H, s), 2.3-2.6 (3H, m), 2.8 (2H, m), 2.9-3.1 (1H, m), 3.3-3.5 (2H, m), 3.6-4.7 (16H, m), 5.06 (1H, d,  $J=3.8\text{Hz}$ ), 5.33 (1H, d,

J=3Hz), 6.77 (2H, d, J=8.6Hz), 6.86 (1H, d, J=8.3Hz), 7.03 (1H, dd, J=8.3Hz and 2Hz), 7.07 (2H, d, J=8.6Hz), 7.31 (1H, d, J=2Hz)

#### Example 47

FR141564 substance was obtained by reacting FR133303 substance with 4-octyloxyphenylsulfonyl chloride according to a similar manner to that of Example 6.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

NMR ( $\text{DMSO-d}_6$  +  $\text{D}_2\text{O}$ ,  $\delta$ ) : 0.87 (3H, t, J=6.7Hz), 0.97 (3H, d, J=6.8Hz), 1.04 (3H, d, J=5.7Hz), 1.1-1.5 (10H, m), 1.6-2.1 (5H, m), 2.45 (3H, m), 2.5-2.7 (1H, m), 3.19 (1H, m), 3.7-4.5 (16H, m), 4.80 (1H, d, J=3Hz), 4.88 (1H, d, J=4Hz), 5.08 (1H, d, J=3Hz), 6.74 (1H, d, J=8.2Hz), 6.82 (1H, d, J=8.2Hz), 6.84 (2H, d, J=8.7Hz), 7.07 (1H, s), 7.51 (2H, d, J=8.7Hz)

FAB-MS  $m/z$  = 1249 (M + Na)

#### Example 48

FR143170 substance was obtained by reacting FR133303 substance with 6-octyloxy-2-naphthylsulfonyl chloride according to a similar manner to that of Example 6.

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

NMR ( $\text{CD}_3\text{OD}$ ,  $\delta$ ) : 0.29 (3H, d, J=6.0Hz), 0.91 (3H, t, J=6.7Hz), 1.07 (3H, d, J=6.9Hz), 1.25-1.6 (10H, m), 1.7-2.2 (5H, m), 2.2-2.6 (4H, m), 3.37 (1H, m), 3.55-4.65 (17H, m), 4.97 (1H, m), 5.54 (1H, m), 6.84 (1H, d, J=8.3Hz), 7.01 (1H, dd, J=8.4Hz and 2Hz), 7.15-7.3 (3H, m), 7.75-8.0 (3H, m), 8.35 (1H, s)

FAB-MS  $m/z$  = 1299 (M + Na)

#### Example 49

To a solution of FR138364 substance obtained in Example 5 (0.24 g) in acetonitrile (5 ml) was added p-toluenesulfonic acid (0.132 g) and stirred for 8 hours at room temperature. The reaction mixture was added to water and the aqueous layer was adjusted to pH 4.5 with saturated sodium bicarbonate aqueous solution. The aqueous solution was subjected to column chromatography on Diaion HP-20 and eluted with 80% aqueous methanol. The fractions containing the object compound were combined and evaporated under reduced pressure to remove methanol. The residue was lyophilized to give FR138912 substance (0.15 g).

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

FAB-MS  $m/z$  = 1272 (M + K)

#### Example 50

The mixture of FR138728 substance obtained in Example 8 (0.15 g) and 1-octyl-1,4-dihydropyridine-4-thione (0.031 g) in N,N-dimethylformamide was stirred for 1.5 hours under ice-cooling. The reaction mixture was pulverized with diethyl ether (50 ml). The precipitate was filtrated and dried over phosphorus pentoxide under reduced pressure. The powder was added to water (300 ml) and adjusted to pH 4.5. The aqueous solution was subjected to column chromatography on Diaion HP-20 (50 ml) and eluted with 80% aqueous methanol. The fractions containing the object compound were combined and evaporated under reduced pressure to remove methanol. The residue was lyophilized to give FR138960 substance (0.15 g).

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

FAB-MS  $m/z$  = 1222 (Free M + Na)

The following compounds (Examples 51 to 53) were obtained according to a similar manner to that of

#### Example 3.

#### Example 51

FR138727 substance

NMR ( $\text{CD}_3\text{OD}$ ,  $\delta$ ) : 0.90 (3H, t, J=6.8Hz), 1.05 (3H, d, J=6.8Hz), 1.17-1.33 (13H, m), 1.6-1.8 (2H, m), 1.9-2.1 (3H, m), 2.50 (1H, m), 2.75 (1H, dd, J=16Hz and 4Hz), 3.40 (1H, m), 3.7-3.8 (1H, m), 3.98 (2H, t, J=6.2Hz), 3.9-4.2 (5H, m), 4.3-4.5 (5H, m), 4.5-4.7 (3H, m), 4.97 (1H, d, J=3Hz), 5.06 (1H, s), 5.20 (1H, d, J=3Hz), 5.40 (1H, d, J=3Hz),



6.85 (1H, d, J=8.3Hz), 6.95 (2H, d, J=8.5Hz), 7.02 (1H, d, J=8.3Hz), 7.30 (1H, d, J=8.5Hz), 7.44 (1H, s)

Example 52

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FR138912 substance  
IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

Example 53

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FR138960 substance  
IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

The following compounds (Preparations 94 and 95) were obtained according to a similar manner to that of Preparation 5.

15

Preparation 94

Succinimido 4-(4-heptyloxyphenyl)benzoate

IR (Nujol) : 1160, 1740, 1600  $\text{cm}^{-1}$

20 NMR ( $\text{CDCl}_3$ ,  $\delta$ ) : 0.87 (3H, t, J=6.8 Hz), 1.2-1.7 (8H, m), 1.7-1.9 (2H, m), 2.92 (4H, s), 4.01 (2H, t, J=6.5 Hz), 7.00 (2H, d, J=8.8 Hz), 7.58 (2H, d, J=8.8 Hz), 7.69 (2H, d, J=8.5 Hz), 8.17 (2H, d, J=8.5 Hz)

Preparation 95

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Succinimido 4-(4-hexyloxyphenoxy)benzoate

IR (Nujol) : 1760, 1720, 1600  $\text{cm}^{-1}$

NMR ( $\text{CDCl}_3$ ,  $\delta$ ) : 0.92 (3H, t, J=6.8 Hz), 1.2-1.5 (6H, m), 1.7-1.9 (2H, m), 2.90 (4H, s), 3.96 (2H, t, J=6.5 Hz), 6.9-7.1 (6H, m), 8.07 (2H, d, J=9 Hz)

30 In the following, the structures of the compounds of Examples 54 and 55 are shown.

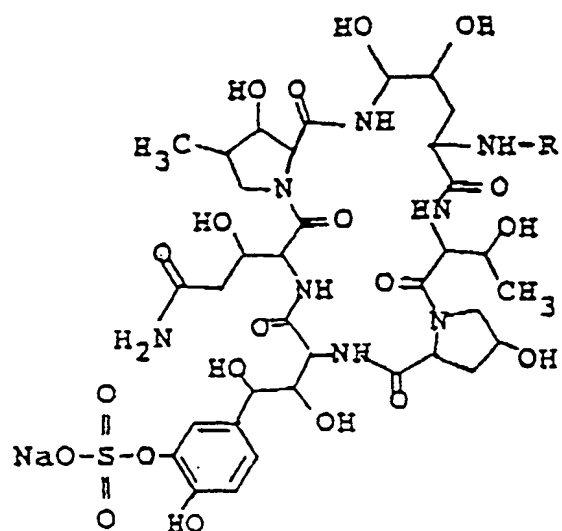
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Example No.	Compound No.	R
54	FR144274	$-\text{CO}-\text{C}_6\text{H}_4-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_6\text{CH}_3$
55	FR144271	$-\text{CO}-\text{C}_6\text{H}_4-\text{O}-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_5\text{CH}_3$

The following compounds (Examples 54 and 55) were obtained according to a similar manner to that of Example 3.

#### Example 54

FR144274

IR (Nujol) : 3300, 1620  $\text{cm}^{-1}$

Anal. Calcd. for  $\text{C}_{55}\text{H}_{73}\text{N}_8\text{SO}_{22}\text{Na} \cdot 6\text{H}_2\text{O}$

C : 48.53, H : 6.29, N : 8.23, S : 2.35

Found C : 48.36, H : 6.34, N : 8.15, S : 2.30

FAB-MS  $m/z$  1275 ( $M+\text{Na}$ )

#### Example 55

FR144271

Anal. Calcd. for  $\text{C}_{54}\text{H}_{71}\text{N}_8\text{SO}_{23}\text{Na} \cdot 6\text{H}_2\text{O}$

C : 47.57, H : 6.14, N : 8.22, S : 2.35

Found C : 47.58, H : 6.05, N : 8.18, S : 2.27

FAB-MS  $m/z$  = 1277 ( $M+\text{Na}$ )

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## 3. A compound of claim 2, wherein

R<sup>1</sup> is lower alkanoyl which may have one or more suitable substituent(s); higher alkanoyl, lower alkenoyl which may have one or more suitable substituent(s); higher alkenoyl; lower alkoxy-carbonyl; higher alkoxy-carbonyl; aryloxy-carbonyl; arylglyoxyloxy; ar(lower)alkoxy-carbonyl which may have one or more suitable substituent(s); lower alkylsulfonyl; arylsulfonyl which may have one or more suitable substituent(s); ar(lower)alkylsulfonyl; or aroyl which may have one or more suitable substituent(s).

## 4. A compound of claim 3, wherein

R<sup>1</sup> is lower alkanoyl; halo(lower)alkanoyl; ar(lower)alkanoyl which may have 1 to 3 suitable substituent(s) selected from the group consisting of hydroxy, lower alkoxy, higher alkoxy, aryl, amino, protected amino, di(lower)alkylamino, lower alkoxyimino and ar(lower)alkoxyimino which may have 1 to 3 higher alkoxy; heterocyclicthio(lower)alkanoyl which may have 1 to 3 higher alkyl; heterocyclic(lower)alkanoyl which may have 1 to 3 suitable substituent(s) selected from the group consisting of lower alkoxyimino, higher alkyl, amino and protected amino; ar(lower)alkoxyimino(lower)alkanoyl which may have 1 to 3 higher alkoxy; higher alkanoyl; ar(lower)alkenoyl which may have 1 to 3 higher alkoxy; higher alkenoyl; lower alkoxy-carbonyl; higher alkoxy-carbonyl; aryloxy-carbonyl; arylsulfonyl which may have 1 to 3 suitable substituent(s) selected from the group consisting of lower alkyl and higher alkoxy; or aroyl which may have 1 to 5 suitable substituent(s) selected from the group consisting of halogen, lower alkyl, higher alkyl, carboxy, lower alkoxy which may have 1 to 10 halogen, lower alkoxy(lower)alkoxy, ar(lower)alkoxy, higher alkoxy which may have 1 to 17 halogen, higher alkenyloxy, aryl which may have 1 to 3 higher alkoxy and aryloxy which may have 1 to 3 lower alkoxy or higher alkoxy.

## 5. A compound of claim 4, wherein

R<sup>1</sup> is lower alkanoyl; halo(lower)alkanoyl; phenyl(lower)alkanoyl or naphthyl(lower)alkanoyl, each of which may have 1 to 3 suitable substituent(s) selected from the group consisting of hydroxy, lower alkoxy, higher alkoxy, phenyl, amino, lower alkoxy-carbonylamino, di(lower)-alkylamino, lower alkoxyimino and phenyl(lower)alkoxyimino which may have 1 to 3 higher alkoxy; pyridylthio(lower)alkanoyl which may have 1 to 3 higher alkyl; imidazolyl(lower)-alkanoyl or thiazolyl(lower)alkanoyl, each of which may have 1 to 3 suitable substituent(s) selected from the group consisting of lower alkoxyimino, higher alkyl, amino and lower alkoxy-carbonylamino; phenyl(lower)alkoxyimino(lower)alkanoyl which may have 1 to 3 higher alkoxy; higher alkanoyl; phenyl(lower)alkenoyl which may have 1 to 3 higher alkoxy; higher alkenoyl; lower alkoxy-carbonyl, higher alkoxy-carbonyl; phenoxycarbonyl; phenylsulfonyl or naphthylsulfonyl, each of which may have 1 to 3 suitable substituent(s) selected from the group consisting of lower alkyl and higher alkoxy; or, benzoyl, naphthoyl or anthrylcarbonyl, each of which may have 1 to 5 suitable substituent(s) selected from the group consisting of halogen, lower alkyl, higher alkyl, carboxy, lower alkoxy which may have 6 to 10 halogen, lower alkoxy(lower)alkoxy, phenyl(lower)alkoxy, higher alkoxy which may have 12 to 17 halogen, higher alkenyloxy, phenyl which may have 1 to 3 higher alkoxy, and phenoxy which may have 1 to 3 lower alkoxy or higher alkoxy.

## 6. A compound of claim 5, wherein

R<sup>1</sup> is phenyl(lower)alkenoyl which may have 1 to 3 higher alkoxy; or benzoyl, naphthoyl or anthrylcarbonyl, each of which may have 1 to 5 suitable substituent(s) selected from the group consisting of halogen, lower alkyl, higher alkyl, carboxy, lower alkoxy which may have 6 to 10 halogen, lower alkoxy(lower)alkoxy, phenyl(lower)alkoxy, higher alkoxy which may have 12 to 17 halogen, higher alkenyloxy, phenyl which may have 1 to 3 higher alkoxy, and phenoxy which may have 1 to 3 lower alkoxy or higher alkoxy.

## 7. A compound of claim 6, wherein

R<sup>1</sup> is phenyl(lower)alkenoyl which may have higher alkoxy; or benzoyl or naphthoyl, each of which may have higher alkoxy, higher alkenyloxy, or phenyl which may have higher alkoxy.

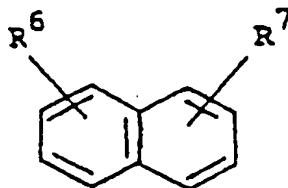
## 8. A compound of claim 7, wherein

R<sup>1</sup> is benzoyl which has higher alkoxy.

9. A compound of claim 8, wherein  
 $R^1$  is 4-octyloxybenzoyl.

10. A compound of claim 7, wherein  
 $R^1$  is phenyl(lower)alkenoyl which has higher alkoxy; or naphthoyl which has higher alkoxy or higher alkenyloxy.

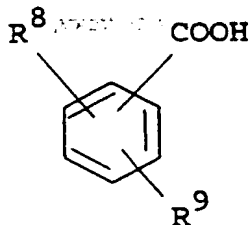
11. A compound of the following formula :



wherein

$R^6$  is ( $C_4$ - $C_6$ )alkoxy, higher alkoxy or higher alkenyloxy, and  
 $R^7$  is  $-COOH$  or  $-SO_3H$ ,  
 or its reactive derivative at the carboxy group or a salt thereof.

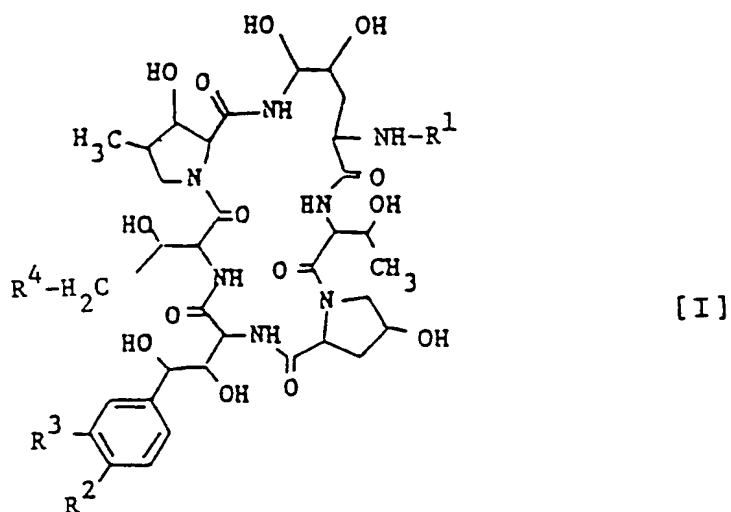
12. A compound of the following formula :



Wherein

$R^8$  is 1 to 4 halogen, and  
 $R^9$  is lower alkoxy which has one or more halogen, higher alkoxy which has one or more halogen,  
 or its reactive derivative at the carboxy group or a salt thereof.

13. A process for the preparation of a polypeptide compound of the formula [I] :



20 wherein

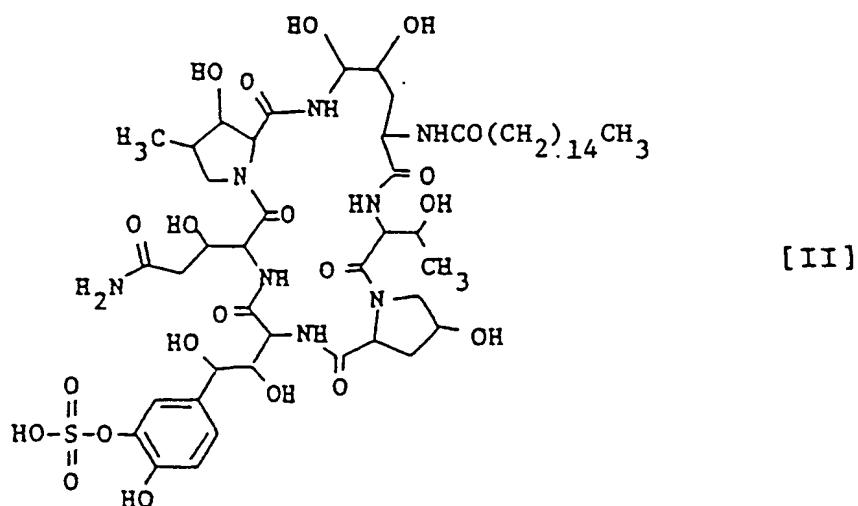
- $R^1$  is hydrogen or acyl group,  
 $R^2$  is hydroxy or acyloxy,  
 $R^3$  is hydrogen or hydroxysulfonyloxy, and  
 $R^4$  is hydrogen or carbamoyl,

25 with proviso that

- (i)  $R^2$  is acyloxy, when  $R^3$  is hydrogen, and  
(ii)  $R^1$  is not palmitoyl, when  $R^2$  is hydroxy,  
 $R^3$  is hydroxysulfonyloxy and  
 $R^4$  is carbamoyl,

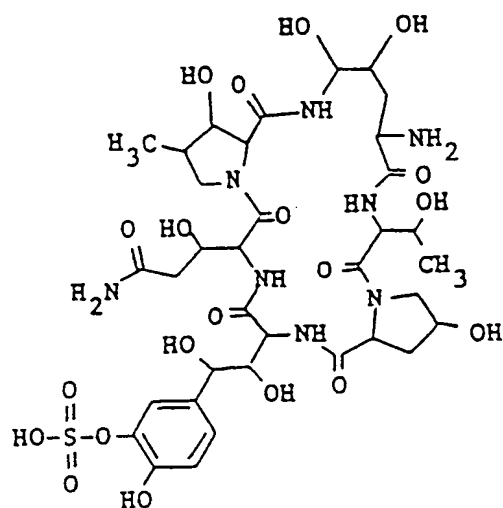
30 or a salt thereof, which comprises

- i) subjecting a compound [II] of the formula :



or a salt thereof,

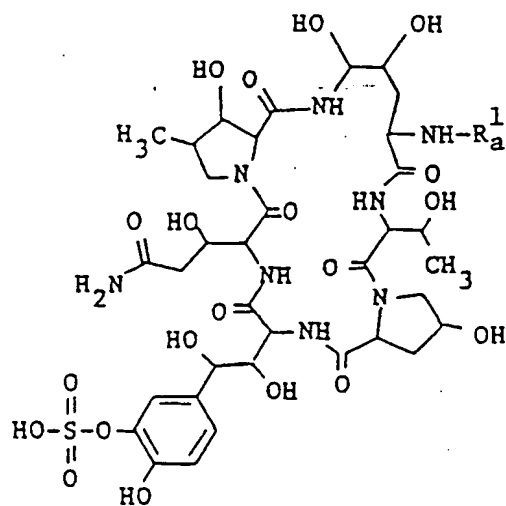
to elimination reaction of N-acyl group, to give a compound of the formula [Ia] :



[Ia]

or a salt thereof, or

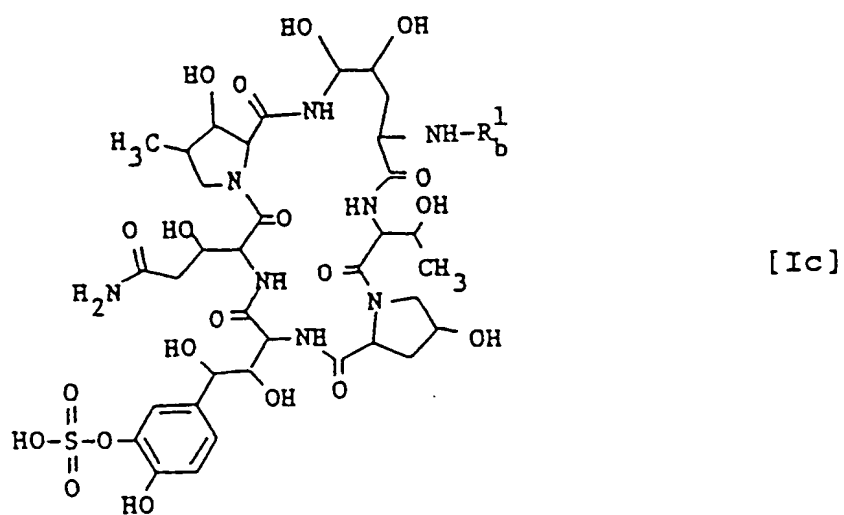
ii) subjecting a compound of [Ia] or a salt thereof thus obtained to acylation reaction, to give a compound of the formula [Ib] :



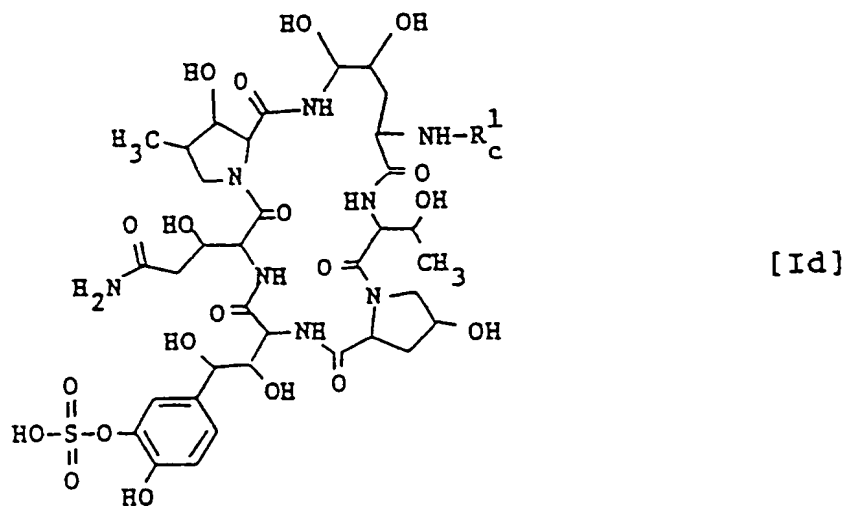
[Ib]

wherein  $R_a^1$  is acyl group exclusive of palmitoyl, or a salt thereof, or

iii) subjecting a compound [Ic] of the formula :



20 wherein  $R_b^1$  is ar(lower)alkanoyl which has higher alkoxy and protected amino, or a salt thereof, to elimination reaction of amino protective group, to give a compound [Id] of the formula :

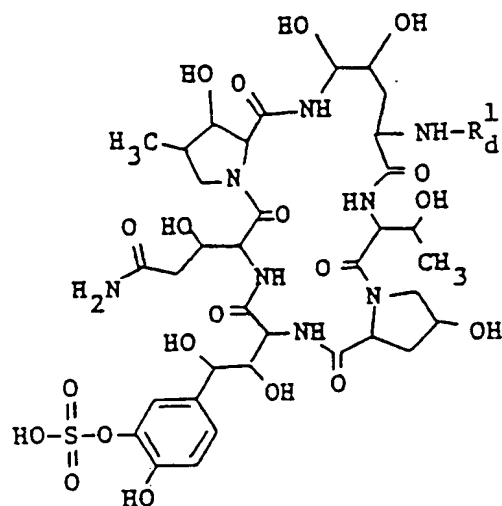


45 wherein  $R_c^1$  is ar(lower)alkanoyl which has higher alkoxy and amino, or a salt thereof, or  
iv) reacting a compound of the formula [Ie] :

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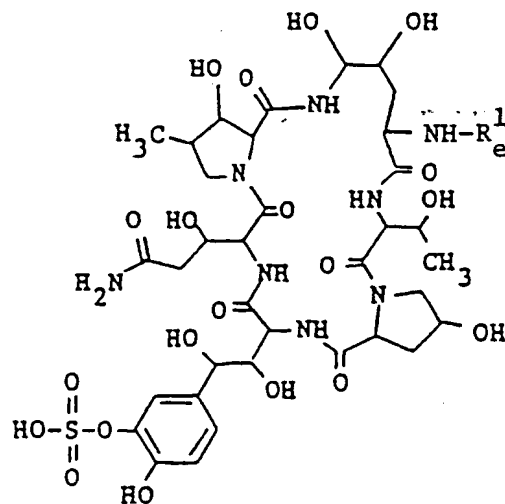
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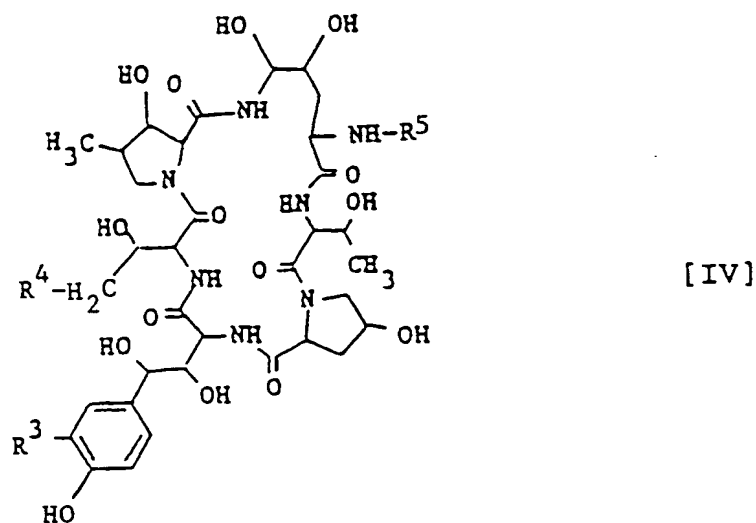
[Ie]

wherein R<sub>d</sub><sup>1</sup> is halo(lower)alkanoyl, or a salt thereof, with pyridinethione which may have higher alkyl or a salt thereof, to give a compound of the formula [If] :



[If]

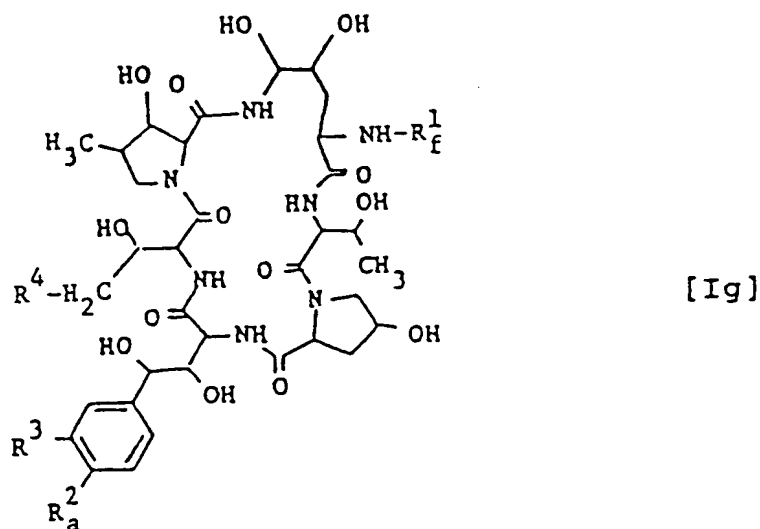
wherein R<sub>e</sub><sup>1</sup> is pyridylthio(lower)alkanoyl which may have higher alkyl, or a salt thereof, or  
v) subjecting a compound of the formula [IV] :



20 wherein

$R^3$  and  $R^4$  are each as defined above, and  
 $R^5$  is acyl group,

or a salt thereof, to acylation reaction, to give a compound of the formula [Ig] :



45 wherein

$R^3$  and  $R^4$  are each as defined above,  
 $R_f^1$  is acyl group, and  
 $R_a^2$  is acyloxy,

or a salt thereof.

50 14. A pharmaceutical composition which comprises, as an active ingredient, a compound of claim 1 or a pharmaceutically acceptable salt thereof in admixture with a pharmaceutically acceptable carrier or excipient.

15. Use of a compound of claim 1 or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for treating or preventing infectious diseases.

16. A compound of claim 1 and a pharmaceutically acceptable salt thereof for use as a medicament.

17. Use of a compound of claim 1 or a pharmaceutically acceptable salt thereof for the manufacture of a medicament.

18. A biologically pure culture of the microorganism *Coelomycetes* strain F-11899 (FERM BP-2635).

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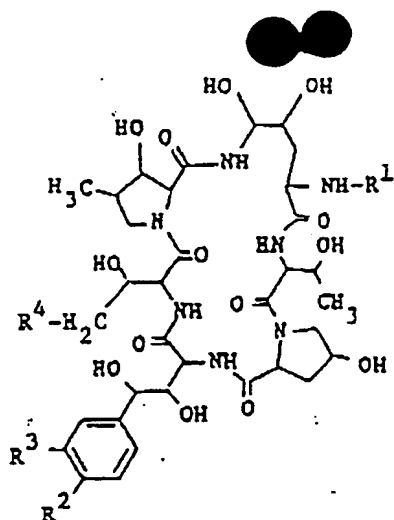
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(54) Cyclic polypeptide with antibiotic activity, process for its preparation and pure culture of a Coelomycetes strain.

(57) A polypeptide compound of the following general formula :



halogen, higher alkoxy which has one or more halogen,  
or its reactive derivative at the carboxy group or a salt thereof. The invention also relates to a biologically pure culture of the microorganism *Coelomycetes* strain F-11899 (FERM BP-2635).

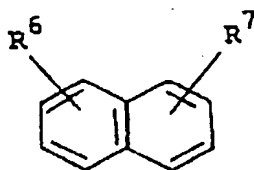
wherein

- R<sup>1</sup> is hydrogen or acyl group,
- R<sup>2</sup> is hydroxy or acyloxy,
- R<sup>3</sup> is hydrogen or hydroxysulfonyloxy, and
- R<sup>4</sup> is hydrogen or carbamoyl,

with proviso that

- (i) R<sup>2</sup> is acyloxy, when R<sup>3</sup> is hydrogen, and
- (ii) R<sup>1</sup> is not palmitoyl, when R<sup>2</sup> is hydroxy, R<sup>3</sup> is hydroxysulfonyloxy and R<sup>4</sup> is carbamoyl,

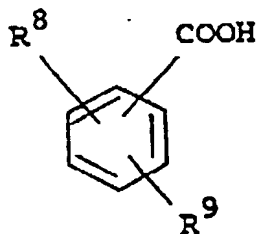
and a pharmaceutically acceptable salt thereof, processes for their preparation and pharmaceutical compositions comprising them. The invention relates also to intermediates of the formula



wherein

- R<sup>6</sup> is (C<sub>4</sub>-C<sub>6</sub>)alkoxy, higher alkoxy or higher alkenyloxy, and
- R<sup>7</sup> is -COOH or -SO<sub>3</sub>H,

or its reactive derivative at the carboxy group or a salt thereof and



Wherein

- R<sup>8</sup> is 1 to 4 halogen, and
- R<sup>9</sup> is lower alkoxy which has one or more



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## EUROPEAN SEARCH REPORT

Application Number

EP 91 10 9856

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
P, X	EP-A-0 431 350 (FUJISAWA PHARMACEUTICAL CO.) * The whole document *	1-5, 14-17	C 07 K 7/56 C 12 P 21/04 C 12 N 1/14 //
P, Y	---	1-10, 13-17	A 61 K 37/02 (C 12 N 1/14 C 12 R 1:645)
Y	EP-A-0 359 529 (MERCK & CO.) * The whole document *	1-10, 13-17	
A	EP-A-0 031 220 (ELI LILLY AND CO.) * The whole document *	1-10, 13-17	
E	EP-A-0 448 354 (MERCK & CO.) * Page 2, line 1 - page 3, line 48 (compound A1); page 11, line 22 - page 13, line 18; examples IV, VII, IX, X; claims 1-10 *	1, 13-17	
			TECHNICAL FIELDS SEARCHED (Int. Cl. 5)
			C 07 K C 12 P C 12 N A 61 K
The present search report has been drawn up for all claims--			
Place of search THE HAGUE		Date of completion of the search 16-04-1992	Examiner GROENENDIJK M.S.M.
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			

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### CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid.
- namely claims:
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

### LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions.

namely:

See sheet -B-

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid.
- namely claims:
- ☒ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

namely claims: 1-10, 13-18

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#### LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims 1-10, 13-18: Polypeptides of the formula of claim 1, their preparation and use.
2. Claim 11: Compounds of the formula of claim 11.
3. Claim 12: Compounds of the formula of claim 12.

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